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Medical Diseases and Obesity in Major Depressive Disorder

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Medical Diseases and Obesity in Major depressive Disorder

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Abstract

The association between major depressive disorder (MDD) and various physical diseases is well recognized. However, previous studies have mainly focused on depression in physically ill individuals. My thesis aims to investigate the reverse direction, i.e. the occurrence of physical diseases including obesity in individuals with MDD. First, I found evidence for higher prevalence rates for eight out of the 16 common physical diseases studied here in depressed people compared to controls with no history of MDD. Affected and unaffected siblings of the depressed subjects showed a similar pattern. The diseases that were significantly more common in depressed subjects were hypertension, hypercholesterolemia, myocardial infarction, asthma, allergic rhinitis/ hay fever, gastric ulcer, osteoarthritis and thyroid disease. In addition, factor and correlational analyses showed that groups of physical diseases tended to cluster together in families where one or more individuals suffered from depression. The most striking of these was a ‘metabolic syndrome’ cluster, which was associated with high body mass index (BMI). Second, I explored personality factors as possible mediators of the link between MDD and physical diseases. Neuroticism, as measured by the Eysenck Personality Questionnaire, was associated with an increased number of self-reported physical diseases in individuals with MDD. Among individual diseases, only asthma had a modest but significant association with MDD. However, we did not find a familial correlation between neuroticism and asthma, suggesting that the phenotypic association between neuroticism and asthma is unlikely to have a genetic component. Third, a new statistical tool was used to estimate the proportion of phenotypic variance of complex diseases/ traits that can be explained by common tag single nucleotide polymorphisms (SNPs), termed “SNP

heritability". Analysis indicated that a substantial proportion of phenotype variance of both BMI and MDD was explained by common genetic variants. There was also suggestive evidence for a substantial genetic correlation between BMI and MDD, although the estimates had large standard errors. This imprecision almost certainly reflects the fact that, although the samples used here were large, even greater sample sizes are required for analyses of SNP heritability. Fourth, I used 32 SNPs identified from a published meta-analysis of genome-wide association studies (GWAS) on BMI to construct both weighted and un-weighted genetic risk scores (GRS) for BMI. Perhaps surprisingly, only 1.27% of the variance of BMI score was explained by the GRSs derived from these SNPs. Subsequent analyses showed that neither GRS alone, nor GRS combined with 'traditional' risk factors, can provide, in our present state of knowledge, a useful tool to discriminate the presence or absence of obesity in depressed people. Fifth, Mendelian randomization (MR) was used to attempt to disentangle the causal relationship between increased BMI and MDD. Although conventional regression analysis suggested a strong association between increased BMI and MDD, MR analysis failed to support the hypothesis that increased BMI is a causal factor in the development of MDD. Finally, I further explored the association between MDD and physical diseases by reviewing published genome wide association study (GWAS) data on MDD to examine whether identified risk loci for MDD overlapped with loci implicated in physical diseases. I then analysed our own GWAS data on depression to examine the presence of case/control differences at loci where there had been physical disease 'hits' in published studies. Analyses indicated that the SNP rs1342326, near the *IL33* gene, which was genome-wide significant in a GWAS on asthma, was over-represented in individuals with MDD. However, MR analysis did not support a causal relationship between suffering from asthma and

having MDD. These results could suggest a single point of genetic overlap between asthma and MDD that might contribute to the observed phenotypic overlap between the two disorders, and highlight the need for further studies in larger samples.

In summary, the analyses presented in this thesis show that the relationship between MDD and physical diseases/ obesity is complicated, but suggests that genetic factors play a role in the overlap between depression and BMI and, by implication, diseases associated with high BMI. Only one non-BMI associated disease, asthma, has an identifiable polymorphism that is also associated with depression and withstands correction for multiple testing. Further larger scale studies searching for disease associated variants are vital in order to understand the pathogenesis of the co-occurrence between MDD and physical diseases/ obesity. These will include genome-wide and, eventually, deep sequencing studies of very large cohorts such as the UK Biobank.

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Statement of Work

The material in my thesis mainly comes from the Munich-GSK depression study and RADIANT study, an umbrella term for three studies comprising the Depression Network (DeNT) study, Depression Case-Control (DeCC) study and the Genome-Based Therapeutic Drugs for Depression (GENDEP) study. The data were made available to the author of this thesis by members of Depression Consortium at the Institute of Psychiatry (Professors Farmer, Lewis, Craig, McGuffin and Dr Breen). Before commencing my PhD, the phenotype and genotype data had already been collected. I was responsible for designing the new analyses, cleaning data, conducting analyses and writing up the thesis under the supervision of my three supervisors (Drs Breen and Davis and Prof McGuffin). In all other respects the work presented in this thesis is original and my own work.

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Chapter One

Introduction

1.1 The definition of major depressive disorder

Major depressive disorder (MDD) is a serious public health problem and causes a huge burden on societies worldwide. The current diagnosis of major depressive disorder relies purely on clinical features, listed as the operational criteria in the Diagnostic and Statistical Manual of Mental Disorder (DSM-IV) (American Psychiatric Association, 2000), and the descriptive and operational criteria of the International Classification of Diseases, Tenth Revision (ICD-10) (Brämer, 1988). At present, no reliable laboratory tests for MDD are available. The diagnosis of major depressive disorder requires several symptoms (Table 1-1) persisting for at least a two-week period. Although the current diagnostic criteria show good reliability for diagnosis (evident in high inter-rater agreement), major depressive disorder may not be a single disease, but rather a heterogeneous disorder with somewhat different clinical manifestations, disease course, and treatment response (Belmaker and Agam, 2008).

Table 1.1 DSM-IV-TR Criteria for Major Depressive Episode

Major depressive episode criterion:

- A. At least five of the following symptoms have been present during the same 2-week period and represent a change from previous functioning: at least one of the symptoms is either 1) depressed mood or 2) loss of interest or pleasure.
 1. Depressed mood most of the day, nearly every day, as indicated either by subjective report (e.g., feels sad and empty) or observation made by others (e.g., appears tearful)
 2. Markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day (as indicated either by subjective account or observation made by others)
 3. Significant weight loss when not dieting or weight gain (e.g., change of more than 5% of body weight in a month), or decrease or increase in appetite nearly every day
 4. Insomnia or hypersomnia nearly every day
 5. Psychomotor agitation or retardation nearly every day (observable by others, not merely subjectively feelings of restlessness or being slowed down)
 6. Fatigue or loss of energy nearly every day
 7. Feelings of worthlessness or inappropriate guilty (which may be delusional) nearly every day (not merely self-reproach or guilt about being sick)
 8. Diminished ability to think or concentrate, or indecisiveness, nearly every day (either by subjective account or as observed by others)
 9. Recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation without a specific plan, or a suicide attempt or specific plan for committing suicide
 - B. The symptoms do not meet criteria for a mixed episode.
 - C. The symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning.
 - D. The symptoms are not due to the direct physiological effects of a substance (e.g. a drug of abuse, a medication) or a general medical condition (e.g. hypothyroidism).
 - E. The symptoms are not better accounted for by bereavement, i.e., after the loss of a loved one, the symptoms persist for longer than 2 months or are characterized by marked functional impairment, morbid preoccupation with worthlessness, suicidal ideation, psychotic symptoms, or psychomotor retardation.
-

1.2 Heterogeneity of major depressive disorder

Although the DSM includes variations on the theme of major depression such as melancholia and depression with a seasonal pattern ('seasonal affective disorder'), DSM IV, and its recent successor DSM 5 essentially take a 'lumper' approach, considering depression to be a single entity. A highly influential advocate of 'lumping' was the Maudsley Hospital's Aubrey Lewis (cited in Kendell, 1977) who proposed that differences between presentations of depression are a matter of degree, rather than of kind. In other words, depression is one 'thing', with quantitative differences in severity, rather than qualitative differences in symptoms or aetiology. However the idea that depression is not one disorder but several, perhaps many different conditions has subsequently been much debated. The clinical observation that depression is very common but also very varied in its presentation has led many to be 'splitters', believing that heterogeneity is inherently likely. As Kendell (1977) has pointed out, the biggest flaw in the 'single entity' proposition is that depression is often secondary to other disorders. Perhaps the most explicit form of 'splitting' based on this phenomenon is the classification system proposed by the Washington University, St Louis school (reviewed by Guze, 1990) in which there are two main categories, primary and secondary depression. Secondary depression is considered to be a broad category that includes any depressive syndrome following major physical illness, other major psychiatric disorders such as schizophrenia, or depression that occurs only during periods of alcohol or other substance abuse. Another important and common category of depression that might be considered as 'secondary' is that following a viral illness such as

glandular fever (White et al., 1998). Here, the prominent feature is often fatigue, which may become chronic, and a substantial proportion of patients fulfil diagnostic criteria for depression.

Another important historical distinction discussed by Kendell (1975) is that of endogenous versus non-endogenous (also called reactive or 'neurotic') depression. Although still viewed as important by many, the concept of endogenous depression was most topical during the 1960s and 70s following the work of Roth and the Newcastle group (cited in Kendell 1977) who used a then relatively novel statistical method, discriminant function analysis, to demonstrate the existence of two major categories based on symptom patterns corresponding to endogenous and neurotic/non endogenous depression. Kendell himself was subsequently unable to replicate the Newcastle group's findings, failing to find what he called a 'point of rarity' between endogenous and non-endogenous depression. Kendell (1977) thus concluded that endogenous and non-endogenous depression are best viewed as opposite ends of a dimension rather than being categorically distinct. The concept of endogenous depression as a somewhat distinct category never quite disappeared however, and it staged a revival in the United States (where it had previously been a less popular concept than in the UK or Europe) in the form of 'melancholic depression' in the 1980s and 90s. The concept of melancholic/endogenous depression was in part supported by the discovery that a proportion of depressed patients fail to show the normal pattern of suppression of blood cortisol levels after being given dexamethasone (Zimmerman et al., 1986). There is a tendency for such patients to show prominent biological symptoms such as early waking, diurnal variation of mood, appetite loss and diminished libido. Notably, the dexamethasone

suppression test (DST) does not appear to be a stable marker, in that in patients who have recurrent depression, the test is positive during some episodes of depression but not others (Coryell et al., 1990). Kendell's (1977) classic review of what he called 'contemporary confusion' in the classification of depression was followed up nearly a decade and a half later by Farmer and McGuffin (1989) who suggested that the evidence, including an absence of stable trait markers, continued to support Kendell's view that the apparent heterogeneity, at least within primary depression, was dimensional rather than categorical. They also considered their own work and that of others relating to adversity and familiarity of patterns of symptomatology. Endogenous and neurotic patterns of depression appear not to 'breed true' in families, and furthermore the symptom pattern of depression following a threatening life event is as likely to be endogenous as reactive/neurotic. Farmer and McGuffin (1989) also concluded that a distinction between familial and non-familial depression is, as with other common diseases, likely to be unhelpful and misleading.

Recent studies have continued to take a dimensional approach to studying heterogeneity in primary depression. One interesting example focused on the first waves of the DeCC and DeNT samples (Korszun et al., 2004). The authors examined clustering of symptomatology within a sample of depressed subjects using exploratory factor analysis, and identified four dimensions that were then supported by confirmatory factor analysis on a different subset of subjects. The factors consisted of (1) core depression plus psychomotor retardation, (2) anxiety, (3) agitation with guilt and suicidality, and (4) appetite gain/hypersomnia. Only the last of these did not show a significant correlation in sib-pairs, suggesting that manifestation of symptom clusters 1-3 may be under

genetic influence. More recently Kendler et al. (2013) applied multivariate genetic analysis in a very large twin sample, where the best model fit was obtained with three genetic (and four environmental) dimensions, which collectively contributed to symptomatology. The genetic dimensions showed some similarities to the familial dimensions of Korzsun et al (2004). In a different analysis of a subset of the data in the present thesis, the GENDEP sample, Uher et al. (2008) used confirmatory factor analysis to test whether depression as measured using three different rating scales to quantify severity can be described by a single dimension. Uher et al. found that a minimum of 3 dimensions was needed to fit the data. Interestingly one of these dimensions resembled endogenous/ non endogenous depression, with higher score being associated with a good response to a tricyclic antidepressant, rather than a selective serotonin reuptake inhibitor.

In summary the evidence to date suggests that, although there is clinical heterogeneity in symptomatology, course and treatment response in MDD, the observed differences are quantitative rather than qualitative. That is, with the probable exceptions of primary/secondary and the bipolar / unipolar depression distinctions that have withstood the test of time (Farmer and McGuffin 1989), depressive disorder is probably a single entity, albeit an amorphous one with multiple dimensions.

1.3 **Epidemiology**

Life time prevalence estimates for major depressive disorder depend crucially on the precise definition that is applied. In population surveys in the USA, the overall lifetime prevalence rates are approximately 12.7% in men and 21.3 % in women (Kessler et al., 1993). However incorporating indicators of severity, such as hospital treatment, reduces estimates of lifetime prevalence markedly to around 4% in men and 8% in women (McGuffin et al., 1996). Whatever the definition, females have almost two-fold higher risk of major depressive disorder compared to males. The mean age of onset for major depressive disorder is approximately 30 years, ranging between the ages of 20 and 50 in most cases (Kessler et al., 2005). Individuals with major depressive disorder tend to have a higher risk of other psychiatric co-morbidities such as anxiety disorder and substance misuse (Belmaker and Agam, 2008).

1.4 Increased mortality in people with major depressive disorder

It is now well established that major psychiatric disorders are associated with excess mortality (Laursen et al., 2007) and the causes can be divided into natural and unnatural. Unnatural causes of death include suicide, homicide and accident, whilst natural causes of death include cardiovascular diseases, malignancy neoplasms, endocrine and metabolic diseases, and other common chronic medical conditions. Both may contribute to higher mortality rates in depressed individuals. Although female patients with MDD have been reported to have a higher relative excess of mortality than males (Laursen et al., 2007) this is not supported by other studies (e.g., Joukamaa et al., 2001). The risk of excess mortality is higher in patients of all ages, with an even higher relative risk of mortality in young- and

middle-age patients (Cuijpers and Smit, 2002). Another striking finding is that even psychiatric healthy people have excess mortality if their first degree relatives have a history of psychiatric admission (Laursen et al., 2007). The results highlight the importance of co-morbidity of physical diseases in individuals with major depressive disorder and their near kin, which have yet been investigated thoroughly.

The excess mortality due to natural causes can be attributed to both genetic and environmental factors. For example, depressed people may be more likely to live in a sedentary and unhealthy life style, fail to exercise and use tobacco or alcohol (Leas and McCabe, 2007). In addition, those with a history of depression have higher rates of overweightness and obesity than healthy controls, leading to a higher risk of various chronic physical diseases (Katon, 2003, Farmer et al., 2008). The excess of natural deaths in depressed individuals and their psychiatric healthy relatives may also be due to genetic factors that contribute to both major depressive disorder and physical diseases (or obesity). If there is genetic correlation between liability to develop major depression and chronic medical diseases or obesity, it might explain why depressed individuals have an excess of natural deaths.

1.5 Physical diseases and major depression

Depression has been found to exert adverse effects on outcome of several physical illnesses, including cardiovascular diseases (Lichtman et al., 2008), cancer (Reich, 2008), and chronic pain related illness (Gatchel et al., 2007).

Although a number of studies have examined the prevalence rates of depression among people with chronic physical illness, fewer studies have investigated the reverse process, i.e. the prevalence rate of physical illness in depressed patients. These studies consistently report higher rates of physical illness in depressed patients.

One large population cross-sectional study (Ortega et al., 2006) found that depression was positively associated with a history of asthma, but not with other physical illness. A meta-analysis (Van der Kooy et al., 2007) showed that clinically diagnosed major depression exerts a high risk for the development of cardiovascular disease, equalling the burden of risk associated with smoking or diabetes. Another study (Carroll et al., 2010) suggested that depression was significantly associated with an increased risk of hypertension in male Vietnam veterans, although the comorbidity of generalized anxiety disorder (GAD) and major depression conferred a greater risk. Using the same dataset, Phillips et al., (2009) found that both GAD and its comorbidity with major depression predict both all-cause and cardiovascular disease mortality. A recent study (Gili et al., 2011) also compared prevalence of medical conditions in patients with recurrent depression and those with first episode depression. Even after controlling for possible confounding factors, all physical illnesses except myocardial infarction, psoriasis and migraine were significantly more prevalent in patients with recurrent depressive episodes compared to those with a single depressive episode.

Longitudinal studies have also addressed the question of whether depression predicts physical illness. Two reviews (Rugulies, 2002, Wulsin and Singal, 2003)

suggested that the relative risk for the onset of coronary heart disease was 1.64 for depressed patients compared to non-depressed people over a 10 year follow-up period. Another study reported that depressed patients had an almost two-thirds higher incidence of physical illness during a 10-year follow-up period, compared to non-depressed people (Holahan et al., 2010). This prospective association between depression and subsequent physical illness was observed for both serious and less serious physical illnesses. Similar effects were reported in another study (Patten et al., 2008), in which higher rates of several physical illnesses, including heart disease, hypertension, asthma, arthritis and migraine were reported in depressed patients.

Several lines of evidence suggest that high body mass index (BMI) and obesity could mediate the relationship between depression and physical illnesses.

Recently, secondary analysis of a large case-control sample provided evidence suggesting that depression is a risk factor for a broad range of physical illnesses, some of which are also related to high BMI (Farmer et al., 2008). The study found that depressed patients had significantly higher rates of a total of 14 physical illnesses compared to healthy controls. Depressed patients were also more likely to be overtly obese than healthy controls (24% v. 10%). In a regression model controlling for age, BMI, and using correction for multiple testing, the associations between depression and myocardial infarction or hypercholesterolaemia were no longer significant. However, even after these adjustments, depressed patients had higher rates of certain illnesses (gastric ulcer, rhinitis, osteoarthritis, thyroid disease, hypertension and asthma).

Several disease patterns of major depression have been shown to impact the association between depression and physical illness, and appear to be related to high BMI. One study (Lamers et al., 2010) used latent class analysis to identify subgroups of patients with depression using symptoms as well as demographic and physical health features. The findings indicated that patients with severe atypical features of depression had more somatic symptoms (e.g. more 'metabolic syndrome', reflecting high BMI and high risk for type II diabetes, coronary heart disease and hypertension), compared to those with a more moderate severity of illness, or those with a severe melancholic sub-type. This finding suggests that metabolic abnormalities play a more prominent role in those with a severe atypical depression subtype. Since there is evidence to suggest that the relationship between physical and depressive illnesses may, at least in part, be related to high BMI/ obesity, the following section will examine the relationship between depression and obesity.

1.6 Definition and epidemiology of obesity

Obesity is defined as abnormal or excessive fat accumulation that may impair health (World Health Organization, 1997). The body mass index (BMI) is a simple measure of body fat based on an individual's height and weight. It is defined as an individual's weight in kilograms divided by the square of their height in meters (kg/m^2). Adults with a BMI between 18.5 and 25 are considered normal weight and people with a BMI between 25 and 30 are overweight, whilst individuals with a BMI equal to 30 or above are obese. Although BMI is an easy to use method to determine body fat, it may over- or under-estimate body fat in

certain populations, such as athletes (Ode et al., 2007). In addition, it does not distinguish whether the body fat comes from intra-abdominal fat or subcutaneous fat.

Obesity is a serious public health problem because, as already mentioned, it is an important risk factor for various physical conditions such as hypertension, type 2 diabetes mellitus, hyperlipidemia, and cardiovascular diseases, which are leading causes of natural deaths in developed countries (Danaei et al., 2009, Willett et al., 2013). The prevalence rate of obesity in the UK rose from 6-8% in 1980, to 23-25% in 2002, with the largest increase in the prevalence of obesity in the younger age group (Rennie and Jebb, 2005). A recent study based on the past trend of obesity in the UK forecasts a further increase its prevalence rate to 41-48% in men, and 35-43% in women, by 2030 (Wang et al., 2011). Obesity is more common in individuals with low educational attainment and low socio-economic status.

1.7 The relationship between depression and obesity

Although some studies (Farmer et al., 2008, Simon et al., 2006) have suggested patients with major depressive disorder are likely to be obese or overweight, other studies (Papakostas et al., 2005, Patten et al., 2009) have not found evidence for this association. A recent meta-analysis (Luppino et al., 2010) found that major depression had only a modest effect on the risk of obesity (OR=1.58). Another study (Blaine, 2008) suggested that depressed patients are more likely to become obese at follow-up than non-depressed people, especially

in female adolescents. Depression and obesity may share common pathways or interact with each other, leading to health complications such as cardiovascular diseases and diabetes (Chapman et al., 2005). The following sections will examine evidence for whether depression predicts obesity, and obesity predicts depression. Factors that may be involved in the relationship between depression and obesity, including demographic, psychosocial and physiological factors, are then considered.

1.7.1 The effects of depression on obesity

In addition to the cross sectional findings reviewed above, longitudinal studies have found evidence to suggest that depressive illness predicts subsequent obesity. A diagnosis of major depression (but not depressive symptoms alone) in adolescents predicts a greater body mass index (BMI) in adult life compared to those who are not depressed (Pine et al., 2001).

Another study (Hasler et al., 2005) also suggested that depression severity showed a dose-response-type relationship with the magnitude of body weight variability. This association was independent of antidepressant use, smoking, physical activity and demographic variables. A recent meta-analysis (Luppino et al., 2010) found that baseline depression (either symptoms or disorder) did not predict being overweight, but did increase the risk of obesity over time.

1.7.2 The effects of obesity on depression

In addition to studies showing that depression predicts subsequent obesity, a

number of studies suggest that obesity may predict subsequent depression. A longitudinal study (Kasen et al., 2008) suggested that young female adults with baseline $BMI \geq 30 \text{ kg/m}^2$ were at a significantly increased risk for subsequent major depression and generalized anxiety disorder. A meta-analysis (Atlantis and Baker, 2008) found that there was a weak level of evidence to suggest that obesity increases the odds of future depression. Ma and Xiao (2010), however, argued that the association between obesity and depression in females is dose-dependent. Several studies (Ma and Xiao, 2010, Dong et al., 2004) also suggested that not only obesity but also chronic medical conditions, race, marital status, age, income and having fewer years of education contribute to depressive symptoms among obese women.

Gender differences may also play a role in moderating the relationship between obesity and depression. For example, in one report, obesity in females was associated with a 37% increase in major depression, whereas obesity in males was associated with a 37% decrease in major depression (Carpenter et al., 2000). Another study (Goldney et al., 2009) also reported that obese men were less likely to have major depression compared to those of normal weight. This may suggest that factors linking obesity and depression are gender specific and involve different mechanisms. However, another study (Simon et al., 2006) suggested that obesity is associated with an increased risk of mood disorders in both genders. Dong et al., (2004) found that only extremely obesity ($BMI \geq 40 \text{ kg/m}^2$) was associated with increased risk of depression across both genders. Having examined evidence for the

relationship between depression and obesity, factors underpinning this association are now considered.

1.8 Psychological and social links between obesity and depression

Socio-demographic and psychosocial factors may influence the relationship between major depressive disorder and obesity. Previous studies (Everson et al., 2002; Gavin et al., 2010) have suggested that socioeconomic status, education and gender may contribute to the co-occurrence of these two disorders. For example, female gender and low level of education have been reported to make people with higher BMI more vulnerable to becoming depressed (Gavin et al., 2010), although not all studies have reached the same conclusion (Everson et al., 2002). Obesity and body image dissatisfaction were associated with depression in people with lower levels of education (<16 years) (Gavin et al., 2010). Whilst low socioeconomic status has been linked to obesity and depression in some studies, one study (Beydoun & Wang, 2010) suggested that socioeconomic status was only inversely associated with body mass index in women through lower food insecurity and reduced physical activity which account for 79% of association between major depression and body mass index.

Several mechanisms, including behavioural, cognitive and psychosocial mechanisms, have been proposed to explain the higher risk of depression in obese people. Two pathways based on possible moderation of the link between obesity and depression have been suggested (Markowitz et al., 2008). First, a “health concern” pathway may lead individuals with morbid obesity to experience

depression because of functional impairment and poor self-rated health. Obese people have more physical disabilities, resulting in impaired physical activity, which is associated with the development of depressive symptoms (Ferraro et al., 2002). Poor self-rated health may affect the development of depression via distorted cognitions, in which people who regard themselves as unhealthy also have more depressogenic beliefs. Second, an “appearance pathway” may lead individuals to become depressed due to body image distortion and dieting. Obese individuals are more likely to be discriminated by other people, leading to lower self-esteem and increased negative affect (Kessler et al., 1999). Such discriminatory experiences may contribute to higher level of depression in obese people. In addition, obesity is associated with body image dissatisfaction. Body image dissatisfaction is further associated with depression. Several studies have shown that body image dissatisfaction mediates the relationship between obesity and depression (Friedman et al., 2002; Gavin et al., 2010). Repeated dieting may cause individuals to feel like failures because they are unable to successfully shed body weight, resulting in depression.

Childhood maltreatment has also been associated with both major depression and obesity, and may play a role in some cases. Both childhood sexual and physical abuse are associated with both depression and obesity in women during middle age, although obesity and depression may be independent outcomes of childhood abuse (i.e. reflecting separate pathophysiological pathways) (Rohde et al., 2008). Another study suggested that childhood neglect but not corporal punishment or psychological aggression is associated with obesity and depression in children

(Whitaker et al., 2007).

1.9 The physiological mechanism linking depression and obesity/ physical illness

Depressed patients are more likely than non-depressed people to experience chronic stress. A proposed physiological mechanism that could mediate the relationship between depression and obesity is via activation of the hypothalamic-pituitary-adrenal (HPA) axis (de Bellis et al., 1993). Elevated cortisol levels, associated with HPA axis dysfunction, have been found in major depressive disorder, especially the melancholic subtype (Gold and Chrousos, 2002). Elevated cortisol levels are also common in obese people. It has been suggested that such elevation is mediated by acute and chronic stress (Seckl and Meaney, 2004). Heightened levels of cortisol have shown to contribute to weight gain, with a particular impact on increased visceral adiposity (Pasquali et al., 2002). Numerous studies have demonstrated that visceral fat (intra-abdominal fat) is associated with components of 'metabolic syndrome' including type 2 diabetes mellitus, hypertension and hyperlipidaemia.

An additional physiological link between depression and physical illness is inflammation. Production of high levels of pro-inflammatory cytokines such as tumour necrosis factor- α (TNF- α), interleukin-1 (IL-1), IL-6 and interferon- γ has been linked with both depression (Kenis and Maes, 2002) and obesity (Miller et al., 2002). It has also been proposed that cytokine production by systemic inflammation acts on the brain to cause depression-like manifestations (Dantzer

et al., 2008). For example, a substantial proportion of patients with hepatitis C receiving interferon treatment develop depression (Asnis and De La Garza, 2006) and major depression is more prevalent in patients with cardiovascular disease, which may in turn be related to chronic inflammation (Lichtman et al., 2008). Depression has been suggested as a maladaptive version of cytokine induced sickness (Dantzer et al., 2008).

High sensitivity C-reactive protein (hsCRP) has been proposed as a biological marker for acute and chronic inflammation. People who reported episodes of major depression within a year before measurement of CRP had increased plasma levels (Surtees et al., 2008). In addition, past-year major depression is a risk factor for subsequent ischemic heart disease. However, hsCRP levels did not account for the association between depression and heart disease. Another study (Kling et al., 2007) further suggested hsCRP and serum amyloid A (SAA) were elevated in remitted and un-medicated women with major depression. This finding suggests that sustained low grade inflammation persisted even after depression remitted, and that it might contribute to development of cardiovascular disease in later life. In addition, IL-6, a potent inducer of the hepatic acute phase response, might play a role in the development of coronary artery disease via metabolic, endothelial and coagulant mechanisms (Yudkin et al., 2000). Psychosocial stress can elevate plasma levels of IL-6 (Zhou et al., 1993), which in turn stimulates the HPA axis, resulting in a tendency towards obesity and cardiovascular disease.

Another physiological pathway that may contribute to the relationship between

depression and obesity is leptin, a peptide hormone, encoded by the obese (OB) gene. Leptin not only acts as an anti-obesity hormone, but can also influence emotion control and cognition (Lu, 2007). Epidemiological studies have reported high plasma levels of leptin in both depression and obesity, suggesting leptin resistance instead of leptin insufficiency in these two conditions (Pasco et al., 2008, Stunkard et al., 2003). One possibility is that leptin resistance serves as a common pathophysiological pathway for the comorbidity of depression and obesity. Other possible physiological mechanisms linking depression and physical illness/ obesity include increased platelet adhesion (Walsh et al., 2002), higher plasma concentration of lipoprotein(a) (Emanuele et al., 2006), and altered autonomic function (Yeragani et al., 2002).

Having reviewed evidence for factors at the psychological, behavioural and physiological levels that could mediate the relationship between depression and obesity/ physical illness, the following section considers the role of genetics in the aetiology of depression and obesity, and genetic overlap between depression and obesity/ physical illness.

1.10 The genetics of major depressive disorder

Family and twin studies have shown that genetic factors contribute to the development of major depressive disorder, with a mean heritability estimate of 37%, but some with studies estimating heritability in excess of 70% (Sullivan et al., 2000). Higher heritability estimates for major depressive disorder are associated with ascertainment in clinical settings (McGuffin et al., 1996) or

incorporation of severity indices in the analysis (Kendler et al., 1993). Numerous candidate gene studies have been conducted to investigate the association between major depression and common genetic polymorphisms. These candidate genes have included serotonin related genes (e.g. *5-HTTLPR*) and brain derived neurotrophic factor gene (*BDNF*). However, the results of these studies have been inconsistent (Lasky-Su et al., 2005, Lohmueller et al., 2003, Levinson, 2006).

One of possible explanation is the lack of consideration of gene environment interaction in these studies. A seminal work by Caspi et al. (2003) has shown that the interaction between the short allele of *5HTTLPR* and stressful life events predicts the development of major depressive disorder. Although one meta-analysis failed to find evidence to support this relationship (Risch et al., 2009), a more recent meta-analysis using a broader range of studies (Karg et al., 2011) found strong evidence for a moderating role of 5-HTTLPR genotype on stress reactivity. As such, the gene-environment interaction may still play a significant role in major depressive disorder (Uher and McGuffin, 2010).

Another genetic approach, genome-wide linkage study (Breen et al., 2011) has identified a locus at chromosome 3p25-26 associated with recurrent major depressive disorder, which has been replicated by independently (Pergadia et al., 2011). However, fine mapping of variants identified in association studies has so far not identified a specific gene contributing to major depressive disorder.

Genome-wide association studies (GWAS) have also provided disappointing results. While genome-wide significant variants have been identified in individual studies (Lewis et al., 2010, Shyn et al., 2011) these have not replicated across studies, and a mega-analysis of GWAS of major depression by the

Psychiatric Genome-wide Consortium (PGC) including more than 25, 000 patients with MDD failed to identify a risk variant reaching genome-wide significance (Major Depressive Disorder Working Group of the Psychiatric GWAS Consortium, 2012; Wray et al., 2012). This is a surprising result considering the twin evidence of moderate to substantial heritability of major depressive disorder.

Possible explanations for the negative GWAS findings include the causal variants being rare, or not tagged in commercial kits; or there being a very high degree of polygenicity, potentially due to common variants of very small effect, which require very large sample sizes. Other explanations include over-estimation of heritability by the twin method, and imprecise classification of the phenotype.

It is therefore of interest that a new analysis tool called genome-wide complex trait analysis (GCTA) has been developed to estimate the proportion of phenotypic variance explained by genome- or chromosome-wide SNPs for complex traits (Yang et al., 2011a) without the necessary assumptions of the twin study method. A recent study (Lubke et al., 2012) using two methods to estimate the SNP-heritability found that 28-32% of phenotypic variance of major depression can be explained by common variants, which is consistent with previous twin studies. The GCTA approach in depression and body mass will be explored later in this thesis.

1.11 The genetics of personality characteristics

Personality characteristics, in particular neuroticism, are thought to confer vulnerability to depression (e.g. Kendler et al., 1993). Personality characteristics have long been considered to have a partly genetic basis (Bouchard Jr and Loehlin, 2001). Twin studies suggest that there is substantial genetic influence on the ‘big five’ personality dimensions of open-mindedness, conscientiousness, extraversion, agreeableness and neuroticism (Jang et al., 1996), ranging from 41% and 61%. In addition, twin studies have also found that there is a substantial genetic overlap between neuroticism and depression, and to a lesser extent, between extraversion and depression (Kendler and Myers, 2010, Middeldorp et al., 2005). Genome-wide linkage analysis (Nash et al., 2004) has indicated two suggestive but not genome-wide significant quantitative trait loci (QTL) in chromosome 1p and chromosome 6p which may confer risk for neuroticism. Genome-wide association studies (Terracciano et al., 2008, Shifman et al., 2008) also failed to identify any locus reaching genome-wide significance for personality traits. The negative results may be attributable to underpowered studies since it is likely that there are genetic variants of small effect on personality characteristics. A meta-analysis of GWAS on personality identified several SNPs responsible for various personality traits in discovery sample but failed to replicate in independent samples (de Moor et al., 2010). One recent study (Vinkhuyzen et al., 2012) using GCTA analysis suggested that 6% of the variance in neuroticism and 12% of extraversion can be explained by common variants. Compared to twin studies, “SNP heritability” provides relatively small estimates of heritability, partly because GCTA only takes common variants into account. Another study (Luciano et al.,

2012) also suggested common genetic effect existed between neuroticism and mood disorder.

1.12 The genetics of obesity

Like neuroticism/ depression, obesity is a complex trait, which is influenced by both genetic and environmental factors. Family and twin studies have estimated the heritability of BMI between 40% and 80% (Stunkard et al., 1986, Allison et al., 1996), and reported that the heritability tends to increase with age (Haworth et al., 2008). Numerous candidate gene studies have been conducted to identify the possible genes contributing to obesity. Associations have been reported with genes encoding leptin, serotonin receptor 2C (*HTR2C*) and dopamine receptor 2 (*DRD2*) genes (McCarthy et al., 2005, Thomas et al., 2000, Mizuta et al., 2008). However, most of candidate gene studies have yielded mixed results, probably due inadequate sample size and possibly confounded by population substructure. Genome-wide association studies have uncovered more risk variants, including the fat mass and obesity-associated (*FTO*) gene, which was robustly associated with obesity (Frayling et al., 2007). This finding has been confirmed in following GWAS studies (Peng et al., 2011). A large meta-analysis including more than two hundred thousand individuals has subsequently identified 32 BMI related loci at genome-wide significance (Speliotes et al., 2010). Although so many risk variants being identified, all of them together explain less than 5% of the variance in BMI. The results of GCTA analysis (Yang et al., 2011b) suggest that 16% of BMI variance is explained by

common genetic variants, suggesting that many of causal variants are just ‘hiding but not missing’, with larger sample sizes needed to discover them.

1.13 Genetic overlap between depression and obesity/ physical illness

As reported above, family and twin studies have shown that genetic factors account for a substantial proportion of the variance of both BMI (Maes et al., 1997) and depression (McGuffin et al., 1996). Although several factors may account for the relationship between obesity and depression, it is possible that a shared common heritable pathogenesis contributes to both medical conditions (McElroy et al., 2004). Comings et al. (1996) first reported that a dinucleotide repeat polymorphism, D7S1875 near the human obesity (OB) gene may contribute to risk for both depression and obesity. Furthermore, a significant association between the BMI and homozygosity for the OB1875 < 208-bp alleles and/or the presence of the DRD2 Taq A1 allele was found across the whole sample, and in women in particular. For women alone these two genes accounted for up to 22.8% of the variance of in BMI. In addition, several candidate genes have been found to associate with both obesity and depression. For example, a study in mice identified two polymorphisms in galanin gene (*GAL*), rs2513280 and rs2513281 which affect expression of *GAL* in the amygdala, which has been found to modulate intake of alcohol and fat, and affect mood, suggesting that differential expression of *GAL* related to common genotypes may confer

risk for the development of obesity, alcoholism and major depression (Davidson et al., 2011).

Another study (McCaffery et al., 2009) used a candidate gene approach to investigate the variants affecting depressive symptoms in coronary heart disease patients. The study suggested that a genetic variant relating to endothelial dysfunction predicts depressive symptoms, which might represent a biological pathway for depression in heart disease patients. Other potential candidate genes with effects on both depression and obesity/ heart disease include *5-HT2A* gene (Rosmond et al., 2002), the serotonin transporter gene (*5HTTLPR*) (Nakatani et al., 2005, Sookoian et al., 2007) and the D2 dopamine receptor gene (*DRD2*) (Spitz et al., 2000).

Recently, genome-wide association studies have revealed some new loci responsible for obesity and heart disease (Thorleifsson et al., 2008, Willer et al., 2008). Many of these loci are the genes that are highly expressed in the brain, particularly in the hypothalamus, which is known to be associated with emotional control. One of these identified genes is brain derived neurotropic factor (*BDNF*) gene, which has been widely reported to play a role in depression and its treatment (Yulug et al., 2010).

These studies provide preliminary evidence to support the hypothesis that some common genes contributing to risk for both depression and obesity/ or cardiovascular disease,. Further studies are needed to deliver a systematic search for genes with pleotropic effects on both depression and obesity/ physical

illnesses.

1.14 Summary

Major depressive disorder is associated with obesity, and various physical diseases, some of which are obesity related. Both major depressive disorder and obesity are prevalent in developed countries and cause a severe disease burden. Although there is a strong association between these two traits, the nature of the relationship remains unclear. Several mechanisms (e.g. psychological, physiological) have been proposed to explain the association of these two traits. In addition, evidence suggests that there may be overlapping mechanisms and aetiology between depression and other physical illnesses, such as those related to inflammation (e.g. cardiovascular disease, asthma).

More research is needed taking a genetic approach to examine whether there is shared genetic risk for depression and obesity/ physical illness. This PhD project investigates the complex relationship between these traits.

1.15 Research questions

The principal issues that will be addressed in this thesis are as follows:

- 1) Are physical diseases more prevalent in siblings as well as those with major depressive disorder themselves, and what is the physical disease pattern in

depressive families? (Chapter 3)

- 2) Do personality traits influence the co-morbidity of physical diseases in individuals with major depression, and in particular, is there any familial correlation between asthma and neuroticism? (Chapter 4)
- 3) What is the 'SNP heritability' of major depression and body mass index, estimated from a large case control study, and is there a genetic correlation between these two traits arising from common variants? (Chapter 5)
- 4) To what extent can genetic risk score constructed by SNPs from a large meta-analysis of GWAS of BMI be used to explain the variation of BMI and the prediction of obesity in individuals with major depressive disorder? (Chapter 6)
- 5) Can Mendelian randomization analysis using instrumental variables disentangle the causal relationship between increased BMI and major depression? (Chapter 7)

Chapter Two

This chapter outlines the characteristics of the samples used in analyses presented in this thesis.

Methods

2. 1 Study population

Throughout this thesis, individuals with major depressive disorder were mainly from RADIANT, an umbrella term for three studies comprising the Depression Network (DeNT) study, the Depression Case-Control (DeCC) study and the Genome-Based Therapeutic Drugs for Depression (GENDEP) study. In addition, data from the Munich depression case control study (which had a very similar in design to DeCC) were used in some analyses. Each study design and inclusion/ exclusion criteria are described as follows and a summary of the design and demographic data for each study is shown in Table 2.1.

2.1.1 Depression Network Study (DeNT)

The DeNT study is a multisite sib-pair genetic linkage study designed to identify genetic loci linked to and/ or associated with susceptibility to recurrent unipolar depression in Caucasian families.

Subjects

Subjects over the age of 18 were identified from various sources including psychiatric clinics, hospitals, general medical practices and from volunteers who responded to advertisements. Full siblings were recruited where two or more had experienced at least two episodes of major depression of at least moderate severity, separated by at least 2 months of remission. The psychiatric diagnoses were made according to the Diagnostic and Statistical Manual 4th edition operational criteria (DSM-IV) or International Classification of Diseases 10th edition operational criteria (ICD-10) for major depressive disorder. They were excluded if one of the siblings fulfilled the criteria of bipolar disorder, schizophrenia, intravenous drug dependence, substance induced mood disorders or experienced mood-incongruent psychotic features. Affected siblings were questioned about the lifetime occurrence of various physical health problems and psychiatric disorders. All subjects were white, of European origin, and recruited from eight different sites: Aarhus, Denmark; Bonn, Germany; Dublin, Ireland; Laussane, Switzerland; St. Louis, USA and Birmingham, Cardiff, and London, UK. Each participant provided written informed consent and these studies were approved by the local ethics committees at each site.

Clinical Assessment

The psychiatric interviews were carried out by graduate psychologists trained to use the Schedules for the Clinical Assessment of Neuropsychiatry (SCAN) (Wing et al., 1990). Diagnoses using the operational criteria of DSM-IV or ICD-10 were generated by a computer-aided interview tool, iShell. The severity and duration of depressive symptoms were rated for the worst and second worst episodes. In order to assess the severity, subjects were asked to identify within each of these episodes of depression a 4-6 week period when they experienced the worst symptoms. All interviewers attended a 4-day SCAN training course and regular subsequent meetings to ensure good inter-rater reliability (the average kappa was coefficient 0.77 (range 0.63-0.89)).

Self-reported questionnaires and other information

To assess the presence of medical diseases, all participants were given a short structured interview to ask whether they had ever been diagnosed or treated by their general practitioner for any of the following 16 physical illness: asthma, diabetes mellitus (type1 or type2), epilepsy, gastric ulcer, hypercholesterolemia, hypertension, kidney disease, liver disease, myocardial infarction, osteoarthritis, osteoporosis, rheumatoid arthritis, allergic rhinitis or hay fever, stroke or thyroid diseases. The selection of physical illness was based on a list of the most common non-communicable, non-malignant diseases in developed countries (Beaglehole and Yach, 2003). Self-reported diagnoses collected in this way have previously been shown to have good agreement with clinical records (Farmer et al., 2008). All participants completed the Eysenck Personality Questionnaires to assess the personality traits of

extraversion, neuroticism, and psychoticism. Body mass index (BMI) was calculated using self-reported height and weight. BMI was defined as weight in kilograms divided by height in meters squared (kg/m^2).

2.1.2 Depression Case control study (DeCC)

The DeCC study is a UK based multi-centre case control study designed to identify genetic variants associated with recurrent major depressive disorder.

Subjects

Individuals with recurrent major depressive disorder were recruited from three clinical UK sites, namely London, Cardiff and Birmingham using the similar methods as the DeNT study to identify the participants from psychiatric clinics, hospitals, general medical practices and from volunteers responding to media advertisements. Individuals were recruited if they were over age of 18 and had experienced two or more major depressive episodes separated by at least 2 months of remission as defined by DSM-IV or ICD-10. The exclusion criteria were the same as for the DeNT study. The psychiatric diagnoses of participants were made by graduate psychologists trained in using SCAN interview, again, as in the DeNT study.

2.1.3 The Genome-Based Therapeutic Drugs for Depression (GENDEP) study

GENDEP is a multi-centre partially randomized pharmacogenetic study aiming to identify the genetic variants associated with response to antidepressant treatment.

Subjects

Individuals with major depressive disorder were recruited from eight European countries, Belgium, Croatia, Denmark, Germany, Italy, Poland, Slovenia and the UK, via clinical referrals, or in response to advertisements. Participants were included if they had experienced at least one major depressive episode of at least moderate severity, according to the operational criteria of DSM-IV or ICD-10. The exclusion criteria were: a first-degree relative with bipolar disorder or schizophrenia, a history of manic or hypomanic episode, mood-incongruent psychotic symptoms, substance misuse, organic affective disorder, current treatment with an antipsychotic or a mood stabilizing medication, pregnancy or lactation. Individuals were also excluded if they had medical contra-indications or adverse reactions to both study medications, namely escitalopram and nortryptiline. Trained psychologists or psychiatrists conducted the clinical interviews using the SCAN interview. In addition to clinical diagnoses, age, sex, self-reported BMI, and measured BMI, which was highly correlated with self-reported BMI, were collected.

2.1.4 GSK-Munich Depression Case Control Study

Munich depression Case Control study was conducted using the same study design as DeCC study in UK.

Subjects

Individuals with recurrent major depressive disorder were recruited at the Max-Planck Institute of Psychiatry in Munich, Germany. Some participants were also recruited from two satellite recruiting hospitals in the Munich area. Experienced research assistants made research diagnoses for psychiatric disorders using the SCAN. Individuals were recruited if they fulfilled the operational criteria for recurrent major depressive disorder of moderate or severe intensity according to DSM-IV or ICD-10. Individuals were excluded if they received the diagnosis of schizophrenia, schizoaffective disorder, bipolar I disorder or any other Axis I diagnosis, except anxiety disorders. Individuals were also excluded if they had experienced mood incongruent psychotic symptoms, a lifetime history of intravenous substance misuse or substance dependence, or depression secondary to medical illnesses or substance/ medication use.

Controls were randomly recruited from a Munich-based community survey. They were screened for the presence of depression or anxiety disorders using the Composite International Diagnostic Screener. Individuals who had no depression or anxiety diagnosis were selected as controls.

The following variables were used in our analyses: age, sex, self-reported BMI and clinical diagnoses.

2.1.5 PsyCoLaus Study

A total of 2,993 participants (1,296 cases and 1,697 controls) were recruited from a psychiatric portion (PsyCoLaus) of a community survey (CoLaus) carried out in Lausanne, Switzerland. A DSM-IV diagnosis of major depressive disorder (MDD) was ascertained using the Diagnostic Interview for Genetics Studies (DIGS) (Nurnberger et al., 1994). Individuals were included as control subjects if they had never fulfilled criteria for MDD. The PsyCoLaus study has been described in more detail elsewhere (Preisig et al., 2009). In brief, the PsyCoLaus study included a semi-structured diagnostic interview and numerous self-rated instruments that evaluated personality traits, attitudes, functioning and sleep patterns. Weight and height were measured at the outpatient clinic at the Centre Hospitalier Universitaire Vaudois (CHUV).

Table 2.1 Summary of demographic data for each study

	RADIANT			GSK- Munich	PsyCoLaus Cases	RADIANT Controls	Munich Controls	PsyCoLaus Controls
	DeNt	DeCC	GENDEP					
Sample Size	1670	1420	706	822	1296	803	857	1698
	47.78	47.39	41.98	51.01	49.69	45.01	51.95	50.59
Age	(9.19)	(12.33)	(11.71)	(13.75)	(8.69)	(12.02)	(13.26)	(8.94)
Female (%)	71%	69%	63%	66%	67%	55%	67%	43%
BMI	26.65 (6.08)		25.52 (4.88)	26.26 (4.89)	25.24 (4.72)	25.11(4.44)	24.57 (4.06)	25.74 (4.34)
Psychiatric Assessment		SCAN			DIGS	PHS	CIDS	
Operational criteria	DSM-IV or ICD-10					N/A		
Inclusion criteria	>= 2 MDE		>=1 MDE	>= 2 MDE		N/A		
Exclusion criteria	1 st degree relative with bipolar I disorder or schizophrenia					N/A		
	Mood-incongruent psychotic symptoms							
	Intravenous drug abuse							
	Secondary depression							
Variables used in analyses								
Age	○	○	○	○	○	○	○	○
Sex	○	○	○	○	○	○	○	○
BMI	○	○	○	○	○	○	○	○
Genotype	○	○	○	○	○	○	○	○
Physical diseases	○	X	X	X	X	X	X	X
EPQ	○	X	X	X	X	X	X	X

DENT: Depression network study; DeCC: Depression Case control study

GENDEP: The Genome-Based Therapeutic Drugs for Depression study

GSK-Munich: GSK Munich depression case control study

BMI: body mass index; EPQ: Eysenck Personality Questionnaire

SCAN: Schedules for Clinical Assessment in Neuropsychiatry

PHS: Past History Schedule; CIDS: Composite International Diagnostic Screener

DIGS: diagnostic interview for genetic studies; MDE: Major depressive episode

2.2 Genotyping and Quality control

All the participants were genotyped using the Illumina HumanHap610-Quad BeadChips (Illumina, Inc., San Diego, CA, USA) produced by the Centre National de Genotypage (CNG), Evry, France. All barcoded DNA samples were processed under full Laboratory Information Management System (LIMS) controls, which ensured stringent quality control.

Individuals were excluded if their >1% of their genotype data was missing, if there was conflicting information regarding genotypic sex assignment and phenotypic data, if they appeared to be related (up to second degree) to other participants, or if they were of non-European ancestry. SNPs with minor allele frequency <1% or showing departure from Hardy-Weinberg equilibrium ($p < 1 \times 10^{-5}$) were excluded from the analyses. Principal component analysis was carried out using EIGENSTRAT after quality control processes were applied.

2.3 Statistical analyses

Analyses conducted for each chapter are outlined below.

Chapter 3 Prevalence and family aggregation of physical diseases

in families with recurrent depression

The analyses for Chapter three used data from the DENT study only. The lifetime prevalence of each medical diseases was compared between cases and controls using mixed-effect logistic regression, controlling for family membership (that is, controlling for the clustered nature of the case data for subjects from the DeNT dataset). Since obesity has been shown to contribute to several medical diseases, we grouped the participants into three categories according to BMI: normal (BMI under 25 kg/m^2), overweight (BMI between 25 kg/m^2 and 30 kg/m^2) and obese (BMI 30 kg/m^2 or over). The BMI categories together with age and sex were also entered into our logistic regression as co-factors. Tetrachoric correlations were used to calculate within-family intra-trait correlation and within-individual cross-trait correlation. Principal components analysis was used to examine the extent to which the 16 disorders tended to co-occur. Principal components analysis with oblique rotation revealed the presence of six components with eigenvalues exceeding 1. All analyses were carried out using STATA, version 12. Statistical significance was defined as $p < 0.05$ and Bonferroni correction was applied. Bonferroni correction should be considered as a conservative method for correcting multiple testing and may incur several problems such as (1) irrelevant null hypothesis, (2) inference defies common sense and (3) increase in type II error (Perneger, 1998). The interpretation should be cautious as it may represent a flout measure of the strength of evidence against the null hypothesis but not an all-or-none measure.

Chapter 4 Neuroticism is associated with asthma and total number of physical disease but no familial correlation exists between neuroticism and asthma

Analyses for Chapter four used data from the DeNT study. The analyses tested whether there were any differences in personality trait scores between the siblings of individuals with recurrent depression and healthy control subjects. Regression analyses were conducted with the following predictor variables: neuroticism, psychoticism, extraversion, age, gender, body mass index and depression status. For simplicity in the analysis, I treated each physical disease (event) as independent although in practice, as I have shown in chapter 3 some physical diseases tend to cluster together. The following outcome variables related to the presence of physical illnesses were used: (1) total number of physical illnesses (poisson regression); (2) any physical illness (logistic regression); (3) a specific physical illness (logistic regression). The *cluster* command was used to group the data by family to take into account the non-independence of the DeNT sibling pairs. All analyses were carried out using STATA, version 12. Statistical significance was defined as $p < 0.05$, and Bonferroni correction was applied. .

For those diseases that remained associated with personality traits after taking into account possible confounding variables, analyses were conducted to investigate whether these diseases and personality traits shared familial affects. This was based on sib-pair modelling derived from the twin modelling approach implemented in the

package Mx (Neale et al., 2006). Typically, twin modelling compares the similarity of monozygotic and dizygotic twins to estimate additive genetic effects (A), shared environmental effects (C) and specific environmental effects (E) (also often referred to as residual effects, since non-shared environment and measurement error are indistinguishable). Familial modelling is a more restricted form of modelling using sib-pair data to estimate the familial influence (F), which combines A and C. It is impossible to calculate the influence of A and C separately, because sib-pair data are like dizygotic twin data, where siblings reared together share on average 50% of their alleles, but 100% of their shared environmental factors. The Cholesky decomposition can describe the extent to which traits share common 'F' influence. As the method requires ordinal data for the analysis, 95% confidence intervals cannot be estimated. The statistical significance was determined by dropping each parameter in turn, and comparing the chi-square of the reduced model to that of the full model, with degrees of freedom calculated as the difference in the number of parameters between the full and reduced models and taking an alpha level of $p < 0.05$. As noted, the analyses were conducted using structural equation modeling in Mx (Neale et al., 2006). To account the selected nature of the subjects, the selection variable (disease status) was included in all models, with prevalence and familiarity parameters fixed (Wood et al., 2011). Since the Mx program cannot include both dichotomous and continuous data in the same analysis, the age- and sex-regressed residuals of neuroticism score was ordinalised into five equal-sized categories. Ordinal data analysis was used to reflect the underlying normal distribution of traits, with one or more thresholds per liability distribution to distinguish between the ordered categories (Rijsdijk et al., 2005).

Chapter 5 Estimation of the genetic correlation between major depressive disorder and body mass index using common genetic variants

Analyses for Chapter five used data from the RADIANT study and GSK-Munich study. Genomewide complex trait analysis (GCTA) (Yang et al., 2011) was used to estimate the genetic relationship matrix (GRM) between individuals, and assess pairwise genomic similarity. Individuals whose relationship to another subject in the dataset was greater than 2.5% (equal to a fourth cousin) were removed to avoid the possible phenotypic similarity between close relatives being due to non-genetic (i.e. shared environmental) effects. The GRM was fitted in a mixed linear model to estimate the proportion of phenotypic variance for BMI and MDD explained by all the autosomal SNPs. For the univariate analysis used to examine which SNPs contribute to variance on BMI or MDD, the variance of a trait or disorder was partitioned into the additive genetic effects of all measured SNPs, and a residual component using a residual maximum likelihood estimation method. For bivariate analysis used to examine which SNPs contribute to variance on both BMI and MDD, the genetic covariance and genetic correlation between the two traits or diseases was estimated, to measure genome-wide pleiotropy (Lee et al., 2012a). Five principal components were used to control for population stratification, and age and sex were included as covariates. The test statistic for the bivariate analysis was obtained by dividing the square of the estimated genetic correlation coefficient by its sampling variance. The p-value was calculated assuming that the test statistic was distributed as a chi-square, with 1 degree of freedom.

Chapter 6 A genetic risk score combining 32 SNPs is associated with body mass index and improves obesity prediction in people with major depressive disorder

Analyses for Chapter six used data from the RADIANT study, GSK-Munich study and PsyCoLaus study.

Selection of SNPs

SNP ‘risk’ alleles associated with increased BMI identified in previous studies were used to construct a 32-SNP additive genetic risk score (GRS) using the approach reported by Speliotes et al. (2010) and Belsky et al. (2012). Of the 32 GRS SNPs, 14 were extracted from GWAS data after applying the quality control measures described above, and another 13 were extracted using proxy SNPs. In addition, 5 SNPs, namely rs11847697, rs11083779, rs11165643, rs7640855, rs1475219 were imputed from 1000 Genomes data. The quality of imputation for these SNPs was checked, and was $>.8$ for all . The call rate for the SNPs was $> 96\%$, with the exception of rs1475219, for which it was approximately 91%. The detailed information for the 32 SNPs was shown in Table 6.1.

Construction of the un-weighted and weighted GRS

To evaluate the cumulative effects of these 32 SNPs on BMI, an additive model was used to construct both un-weighted and weighted genetic risk scores. The un-weighted GRS (uGRS) was calculated by summation of the number of risk alleles across the 32 variants (Table 2.2). The weighted GRS (wGRS) was calculated by multiplying the number of risk alleles at each locus (0, 1, 2) by the corresponding effect sizes, in kg/m^2 per allele, as reported by Speliotes et al. (2010), and then summing the products. In order to reduce bias, only the participants without any missing data were included in our GRS analysis.

Table 2.2. Single Nucleotide Polymorphisms Included in the Genetic Risk Score in RADIANT study

Chr	Nearest Gene	SNP name	Alleles	BMI- increasing Allele	Frequency of BMI- increasing allele	GWAS Effect-Size for BMI	Call rate
1	NEGR1	rs2568958	A/G	A	62.5%	0.13	99.95%
	TNNI3K	rs1514175	A/G	A	42.3%	0.07	99.86%
	PTBP2	rs11165643	C/T	T	58.8%	0.06	99.07%
	SEC16B	rs10913469	C/T	C	19.2%	0.22	100%
2	TMEM18	rs2867125	C/T	C	82.9%	0.31	99.98%
	ADCY3,RBJ	rs10182181	A/G	G	46.9%	0.14	99.40%
	FANCL	rs759250	A/G	A	28.4%	0.1	100%
	LRP1B	rs6714473	C/T	T	9.7%	0.09	99.85%
3	CADM2	rs7640855	A/G	A	19.0%	0.1	96.83%
	ETV5	rs7647305	C/T	C	79.0%	0.14	99.93%
4	GNPDA2	rs12641981	C/T	T	44.1%	0.18	100%
	SLC39A8	rs13107325	C/T	T	7.5%	0.19	99.91%
5	FLJ35779	rs253414	C/T	T	66.4%	0.1	99.93%
	ZNF608	rs6864049	A/G	A	47.2%	0.07	100%
6	TFAP2B	rs987237	A/G	A	18.2%	0.13	100%
	NUDT3	rs206936	A/G	G	18.0%	0.06	95.99%
9	LRRN6C	rs2183825	C/T	C	32.9%	0.11	99.98%
11	STK33, RPL27A	rs10840065	A/G	A	51.6%	0.06	100%
	BDNF	rs6265	C/T	C	79.8%	0.19	100%
	MTCH2	rs10838738	A/G	G	34.5%	0.06	100%
12	BCDIN3, FAIM2	rs7138803	A/G	A	37.5%	0.12	100%
13	MTIF3	rs1475219	C/T	C	20.4%	0.09	90.61%

14	PRKD1	rs11847697	C/T	T	3.6%	0.17	96.87%
	NRXN3	rs10146997	A/G	G	21.9%	0.13	100%
15	MAP2K5	rs2241423	A/G	G	77.2%	0.13	99.96%
16	GPRC5B	rs12446632	A/G	G	86.1%	0.17	99.93%
	SH2B1	rs4788102	A/G	A	39.0%	0.15	100%
	FTO	rs3751812	G/T	T	41.0%	0.39	100%
18	MC4R	rs921971	C/T	C	26.6%	0.23	99.98%
19	KCTD15	rs29941	A/G	G	68.3%	0.06	100%
	ZC3H4, TMEM160	rs2303108	C/T	C	71.4%	0.09	100%
	QPCTL	rs11083779	C/T	T	95.8%	0.15	98.28%

Regression and ROC models

A linear regression model was calculated to predict BMI. Predictor variables included the ‘known’ risk factors for depression (as found in Chapter 3) and ‘traditional’ risk factors (age, sex and principal components of ancestry), as well as the GRS. Since the BMI data did not follow a normal distribution, natural log-transformed BMI was used for the analyses. Logistic regression, adjusted by age, sex, depression status and ancestry, was used to predict the probability of obesity with each model. Receiver-operating characteristic (ROC) curve analysis was conducted to calculate the area under the curve (AUC), to evaluate how well the model could discriminate between obesity and non-obesity. First, the difference between AUCs for models incorporating traditional risk factors (age, sex and ancestry) with and without GRS was compared. Subsequently, models comprising wGRS only, and models incorporating other risk factors (e.g. age, sex) were compared. To control for the possible presence of population stratification, all analyses were adjusted for the first five principal components of ancestry, which were calculated with EIGENSOFT (Price et al., 2006). All data were analysed using STATA version 12.1 (STATA Corp, Texas). Two-tailed values of $p < 0.05$ were considered significant.

Chapter 7 Relationship between obesity and the risk of clinically significant depression: Mendelian randomisation study

Analyses for Chapter 7 used data from RADIANT study and GSK-Munich study.

Construction of the weighted GRS (wGRS)

Risk alleles were defined as alleles associated with an increased risk of BMI. As described above, an additive genetic risk score (GRS) was derived from the 32 SNPs reported by Speliotes et al., (2010) including rs3751812 of *FTO* gene. As in Chapter 6, we have shown GRS explained a modest but significant proportion of BMI. In order to reduce the bias caused by missing data, only the participants without any missing data were included in our MR analysis while using weighted genetic risk score (wGRS) as the instrument.

Statistical analysis

Prior to the analysis using the GRS, the observed increase in risk of major depression per 1kg/m² change in BMI was assessed using probit regression, after controlling for age, sex and principal components of ancestry. The association between (1) *FTO* genotype and BMI, and (2) wGRS and BMI was analyzed using linear regression, with the covariates mentioned above. The predictor variable, *FTO* genotype, was modelled in an additive model (i.e. 0, 1, 2 copies of risk alleles). F statistics derived from the first-stage regression were used to evaluate the predictive strength of the variables (*FTO* genotype and wGRS), with values greater than 10 indicating a strong instrument suitable for instrumental-variables regression. We then performed an instrumental-variables regression analysis using the *ivprobit* command in STATA, to examine whether *FTO* genotype or wGRS was associated with major depression

through their associations with BMI. To correct for the possible presence of population stratification, all analyses were adjusted for the first five principal components of ancestry which were calculated with EIGENSOFT (Price et al., 2006). All the analyses were conducted using STATA version 12.1 (StataCorp., College Station, TX).

Chapter 8 Further exploration of the associations between physical diseases and major depression

Chapter 8 examined whether individual polymorphisms associated with physical diseases are overrepresented in our sample of depressed individuals. A GRS for asthma was then calculated, based on findings from Chapter 3 suggesting that asthma was more prevalent and showed familial aggregation with MDD. Finally, an analysis using Mendelian Randomization (MR) was conducted, given that the analysis of GWAS data suggested that the phenotypic overlap of asthma and MDD may have a molecular genetic underpinning. The motivation for the MR analysis is that the interpretation of an association between a modifiable risk factor and a disease found in traditional epidemiological studies is potentially limited by confounding factors, resulting in lack of ability to make causal inference about the association between a risk factor and a disease. The principle of MR analysis (Smith and Ebrahim, 2003) is to use genetic variants contributing to the levels of a possible risk factor for a disease to examine the causal effect between levels of that risk factor (as reflected in number of risk alleles) and disease risk (Sheehan et al., 2008). The random assortment of genetic variants from parents to offspring at meiosis provides a relatively robust

method for assessing the causal nature of environmental risk factors, because the assortment of risk alleles is independent of confounding factors.

Method 1

First, all Genome-wide association studies (GWAS) on body mass index (BMI), obesity, asthma, allergic rhinitis, hypertension and myocardial infarction were searched using publications in National Human Genome Research Institute (<http://www.genome.gov/gwastudies/>). Hypercholesterolemia was not included in the search, because the published GWAS on cholesterol are separated into studies on HDL(High Density Lipoprotein)-cholesterol and LDL (Low Density Lipoprotein)-cholesterol as a continuous variable, and not, as in our data, the disease status of hypercholesterolemia (present/absent). Second, all SNPs with p values $> 5 \times 10^{-8}$ in the initial GWAS reports were dropped, because we wanted to include genetic variants that are reliably associated with physical illnesses only. Third, reports on independent samples were examined to see whether hits reported in published studies were replicated. If associations were not replicated, the Medline database was searched to find whether the same hits had been reported in any other papers. Fourth, candidate SNPs found in GWAS studies were examined in GWAS data sets from Psychiatric Genetic Consortium (PGC)- Major depressive disorder (MDD) (<http://www.broadinstitute.org/mpg/ricopili/>) to see whether these genetic polymorphisms were over-represented in patients with MDD. The aim of the Psychiatric Genomics Consortium (PGC) is to conduct meta- and mega-analyses of genome-wide genomic data for various psychiatric disorders by uniting the investigators around the world. The PGC was established in early 2007 and now includes more than 800 investigators from 38 countries. Currently over 900,000

individuals were recruited for analyses. Initially, the PGC focused on five major psychiatric disorders, namely autism, attention-deficit hyperactivity disorder, schizophrenia, bipolar disorder and major depressive disorder and now other important psychiatric diseases were included as well.

When a candidate SNP was not present on a particular genotyping array used in depression studies, proxy SNPs in linkage disequilibrium (LD) with that candidate SNP were identified using SNP annotation and proxy search, with the r^2 threshold set at 0.8 (<http://www.broadinstitute.org/mpg/snap/>). Fifth, all of the remaining SNPs in the same region were compared with each other to identify those that were in linkage disequilibrium, and two SNPs with LD > 0.5 were considered as the same hit. Finally, correction for multiple testing using the Bonferroni method was applied. The detailed working flow is shown in Figure 2.1.

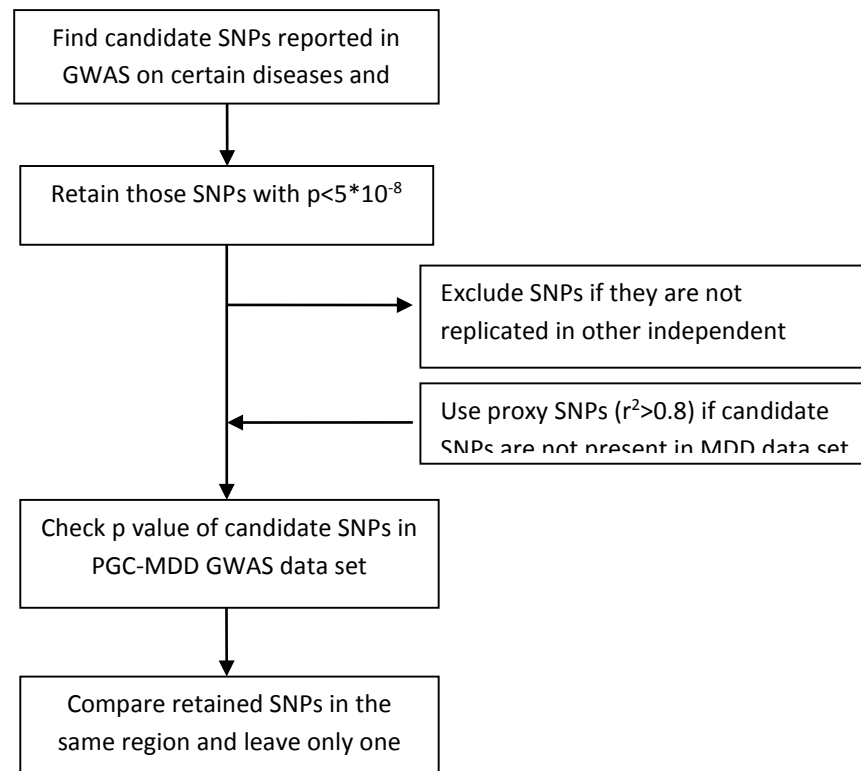


Figure 2.1 Working flow chart for method 1

Method 2

After identifying rs1342326, which is reliably associated with asthma, and was significantly over-represented in MDD based on data from the Psychiatric Genetic Consortium (PGC)- Major depressive disorder (MDD) (<http://www.broadinstitute.org/mpg/ricopili/>), a Mendelian randomisation analysis was conducted using our RADIANT sample to see whether we could confirm this association. The details of participants and phenotype information in RADIANT are described in Table 8.2. Again, two instrumental variables (IVs) were used for the Mendelian randomisation analysis. The first IV was rs1342326, identified in the analysis described in Method 1. The second IV was a GRS for asthma, which was constructed as follows. First, published GWAS studies on asthma conducted before October, 2012 were searched. Seventeen SNPs, including rs1342326, were found to fulfil our requirements. Of the 17 SNPs, 12 SNPs were extracted from GWAS data after applying stringent quality control measures. The other four SNPs were extracted using proxy SNPs. One SNP, rs4129267, was not available in our GWAS data, and proxy SNPs could not be identified. As such, this allele was excluded. Subsequently, another four SNPs (rs9807989, rs2381416, rs11078927 and rs4794820) were excluded due to high linkage disequilibrium with other identified SNPs (r^2 value between 0.58 and 0.91). The call rates for all of these twelve SNPs was > 99% (Table 7.1). An additive model was applied to construct a weighted GRS using the 12 SNPs. The weighted GRS (wGRS) was calculated by multiplying the number of risk alleles at each locus (0, 1, 2) with the corresponding effect size as reported in each GWAS study, and then summing the products. Only participants without any missing data were included in the GRS analysis.

Logistic regression was then used to investigate the relationship between asthma and MDD after controlling age, sex and principal components of ancestry. The association between rs1342326 genotype/ wGRS and asthma was then analysed using logistic regression, with inclusion of the covariates mentioned above. The predictor variable *rs1342326* genotype was modelled with an additive model (i.e. 0, 1, 2 copies of risk alleles). As in Chapter 7, F statistics from the first-stage regression were used to evaluate the strength of the instrumental variable, with values > 10 indicating a strong instrument suitable for instrumental-variables regression. An instrumental-variables regression analysis was then performed using the *ivprobit* command in STATA to examine whether rs1342326 genotype or the wGRS was associated with major depression through their association with asthma. To correct for the possible presence of population stratification, all analyses were adjusted for the first five principal components of ancestry which were calculated with EIGENSOFT (Price et al., 2006). All the analyses were conducted using STATA version 12.1 (StataCorp., College Station, TX).

Chapter Three

Prevalence and family aggregation of physical diseases in families with recurrent depression

Abstract

As described in Chapter 1, there is accumulating evidence that certain common physical diseases are associated with major depression, but the causal underpinnings of such associations remain unclear. This chapter describes a study that aimed to replicate previous work at the MRC SGDP Centre comparing the prevalence of physical diseases in depressed patients and psychiatrically healthy controls. A further study investigating familial aggregation of physical diseases and their inter-correlation in 721 families with recurrent depression is also described.

After controlling for age, gender, body mass index (BMI), family membership, and having applied correction for multiple comparisons, the following disorders were significantly more frequent in depressed patients: hypertension, hypercholesterolemia, myocardial infarction, asthma, allergic rhinitis/ hay fever, gastric ulcer, osteoarthritis and thyroid disease. Hypertension, hypercholesterolemia, asthma, osteoarthritis and gastric ulcer appeared to cluster in families in depressed patients. Principal components analysis of the 16 diseases conducted identified six groups of disorders, with the most significant component relating to a 'metabolic

syndrome' group, consisting of type 2 diabetes, hypertension, hypercholesterolemia and myocardial infarction. These findings confirmed that patients with recurrent depression have substantially increased rates of several common physical diseases, and suggest that familial aggregation of such diseases is significant. Common physiological pathways linking depression and physical diseases (e.g. via hypothalamus-pituitary axis dysfunction and/or inflammatory processes) might explain the observed comorbidity and familiarity.

3.1 Introduction

Major depression is a serious public health problem in its own right, but there is also emerging evidence of its co-occurrence with various chronic medical disorders (Farmer et al., 2008). According to World Health Organization's report (WHO, 2008), unipolar depressive disorder will become the top leading cause of burden of disease in 2030, followed by ischemic heart disease. The disability-adjusted life years (DALYs) is a measure of overall disease burden, expressed as the number of year lost because of illness, disability or premature death, and unipolar depression comprises 10% of total DALYs in high-income countries. Comorbidity of major depression and chronic medical disorders significantly influence the life quality and impact the DALYs even more.

Chapter 1 presented evidence suggesting that there is a substantial co-occurrence between MDD and various chronic medical disorders (Farmer et al., 2008), and that the relationship between major depression and chronic medical disorders is likely to be bi-directional. On one hand, major depression can influence the occurrence or progression of medical disorders via biological mechanisms such as increased inflammation and autonomic dysfunction (Dantzer et al., 2008, Koschke et al., 2009). In addition, major depression is associated with various risk factors related to chronic medical disorders, including cigarette smoking, alcohol misuse, sedentary life style, poor adherence to treatment and obesity (Hämäläinen et al., 2001). On the other hand, poor physical health is likely to increase the risk of onset or persistence of depression (Harris et al., 2006).

Chapter 1 also highlighted the high rates of co-morbidity between certain physical diseases and depression (Gatchel et al., 2007, Lichtman et al., 2008). For example, up to one third of people developed major or minor depression following acute myocardial infarction (MI) during one-year follow-up (Strik et al., 2004) and patients with moderate to severe post-MI depression have a higher risk of five-year mortality (Lespérance et al., 2002). In particular, previous work from our group has shown higher rates of common physical diseases and obesity in patients with recurrent depression compared to healthy controls (Farmer et al., 2008). Not only case-control studies but also population-based studies showed people with major depression have a higher rate of various medical diseases (Wu et al., 2012, Chien et al., 2013). Elsewhere, it has been shown that depressed patients have a higher likelihood of experiencing physical illness during a follow-up period compared to community controls (Holahan et al., 2010) and that depressed patients with comorbid physical illness tend to have an increased health care cost (Creed et al., 2002), a poorer quality of life (Small et al., 1996), poor medical compliance (DiMatteo et al., 2000) and increased all-cause mortality (Axon et al., 2010).

Previous studies have shown that many common diseases are familial, for example being more prevalent in siblings of individuals with those diseases than in the general population. It seems likely that the same pattern should be seen in families of patients with depression. That is, if causal pathways implicated in depression and physical illness are shared, co-existent physical diseases should also be seen more commonly in siblings of depressed patients. Such a finding would have implications for both general practitioners and psychiatrists looking after depressed patients and their relatives.

Principal component analysis (PCA) is a variable reduction technique used to reduce the number of highly correlated observed variables to a smaller number of principal components, which account for a certain proportion of the variance of the observed variables. Evidence has shown that a number of physical diseases are correlated with each other, suggesting that they may have similar mechanisms of pathogenesis. PCA has the advantage of being able to detect latent disease structure, allowing us to detect possible common underlying pathways for certain clusters of medical diseases.

Here, we aimed to replicate previous findings from the DeCC study (Farmer et al., 2008) of increased rates of physical diseases in patients with recurrent major depression, and to explore the phenomenon further by assessing familiarity in depressed siblings. We also examined the extent to which groups of physical disorders clustered together across a sample of depressed individuals. The decision was taken not to include migraine in these analyses because the co-occurrence of migraine and depression within the DeCC and DeNt samples has been extensively explored in another PhD thesis, and in publications arising from it (e.g. Samaan et al., 2009; Ball et al., 2009)

3.2 Methods

The cases of recurrent depression used in this study were from the Depression Network (DeNT) Study. The details of the sampling, clinical assessments, measures and statistics are described in Chapter 2.

3.3 Results

A total of 1670 participants with recurrent depression and 85 unaffected siblings were recruited from 721 families. Their medical history interview data was compared with that from 803 healthy controls. Seventy-four per cent of the case group were women, compared with 55 % of the control group ($\chi^2=87.26$, d.f.=1, $p<0.001$). The mean age at interview of the case group was higher than control group (47.78 (S.D.=9.19) vs 45.01 (S.D.=12.00), $t=6.344$, d.f.=2009.85, $p<0.001$). The median age across all subjects was 46. Twenty four per cent of the case group was obese, compared with 10% of the control group ($\chi^2=68.52$, d.f.=1, $p<0.001$), and 48% of cases and 58% of controls were within the normal weight range.

Lifetime prevalence rates of common medical diseases stratified by group are shown in Table 3.1. Nine out of 16 diseases were more common in depressed patients compared to healthy controls, after controlling for family membership. The medical diseases with the highest prevalence rates in the case and control groups were asthma, hypertension and hypercholesterolemia. Differences in disease prevalence between cases and controls were assessed using mixed-effect logistic regression, with the explanatory variables being depression status (case or control), BMI, gender, age and family membership. The Bonferroni method was used to correct for multiple comparisons, resulting in a critical p value of 0.003125 (0.05/16). The pattern of higher prevalence rates of various physical diseases was observed not only in depressed probands, but also in their unaffected siblings for five diseases. Table 3.2 shows that eight diseases remained significantly more common in depressed patients after adjusting for BMI, gender, age and family membership: namely asthma, gastric

ulcer, hypercholesterolemia, hypertension, myocardial infarction, osteoarthritis, rhinitis/hay fever and thyroid disease (odds ratios ranged between 2.37 and 5.81). Asthma (33.3% vs. 15.8%) and thyroid diseases (10.3% vs. 2.8%) were more prevalent in female depressed patients than male depressed patients, whereas hypercholesterolemia was more common in males than females (17.9% vs. 11.0%). We did not find an interaction effect between sex and depression for any physical diseases with the exception of asthma (prevalence: 6.9% in male controls, 12.5% in female controls, 15.8% in male patients and 33.3% in female patients, $p<0.01$).

Although some physical diseases are more prevalent with increasing age, our results are unlikely to have being influenced by an unequal proportion of older (>55 years) participants among the depressed sample compared to the healthy controls (21.7% v. 24.5%. $\chi^2=2.41$, $p=0.12$). Apart from a higher rate of asthma in the UK sites (34.8% v. 23.3%, $p<0.001$), the prevalence rate for each medical disease was not significantly different between UK and non-UK sites. Although the prevalence rates of various physical diseases in unaffected siblings was similar to that in depressed probands, only hypercholesterolemia was significantly more prevalent in unaffected siblings than in healthy controls after adjusting for the effects of age, gender, BMI and family membership, and using Bonferroni correction (Table 3.2).

Table 3.3 shows familial intra-trait correlation in sibling pairs affected by depression. The findings suggest that hypertension, hypercholesterolemia, asthma, osteoarthritis and gastric ulcer are familial in depressed patients. Table 3.4 shows the results of principal component analysis used to examine the inter-relationship of the 16 diseases among the depressed patients. Six components were extracted, taking a cut-

off eigenvalue of 1.0 or more. The six components explained a total of 47.7% of the variance, with component1 contributing 12.9%, the remaining five components contributing 6.3% to 7.6%. Although component 5 appeared to only tap variance relating to one variable, type I diabetes mellitus, this could be attributed to the fact that (1) underlying components were inferred from the correlation among all variables and each factor is estimated as the weighted sum of each variables; and (2) type I diabetes usually develops in childhood, and therefore may be more weakly related to other common medical diseases with an onset in mid-adulthood. Substantial correlations between sibling pairs for components1, 2 and 4 were observed, ranging between 0.13 and 0.24.

Table 3.1 Lifetime prevalence rates of self-reported medical diseases

Medical Disorder	Life time prevalence (%)			DC vs. controls	US vs. controls
	Depressed	Unaffected	Controls	OR (95% CI)	OR (95% CI)
	Cases (DC)	sibs (US)		<i>P</i>	<i>P</i>
N	1670	85	803		
Asthma	28.7	14.3	9.3	3.89(3.01~5.03)	1.61 (0.85~3.05)
				<0.001*	0.145
Diabetes type 1	0.7	0	1.1	0.61(0.27~1.40)	---
				0.25	
Diabetes type 2	2.6	3.5	1.4	1.84(0.96~3.52)	2.53 (0.71~9.01)
				0.07	0.15
Epilepsy	2.1	0	0.4	6.12(1.88~19.95)	---
				0.003*	
Gastric ulcer	7.3	6.0	1.1	6.72(3.50~12.89)	5.28
				<0.001*	(1.79~15.54)
					0.003*
High cholesterol	13.2	24.1	5.7	2.22(1.62~3.05)	4.63 (2.64~8.12)
				<0.001*	<0.001*
Hypertension	15.1	22.0	6.8	2.33(1.72~3.14)	3.68 (1.93~6.99)
				<0.001*	<0.001*
Kidney disease	2.1	1.2	1.0	1.95(0.94~4.08)	1.10 (0.14~8.46)
				0.07	0.93
Liver disease	2.0	2.4	0.7	2.15(1.00~4.65)	2.52
				0.05	(0.52~12.14)
					0.25
Myocardial	4.4	5.9	1.7	2.67(1.49~4.78)	3.67
infarction				0.001*	(1.29~10.44)
					0.015
Osteoarthritis	11.0	14.5	3.5	2.94(2.01~4.30)	4.01 (1.95~8.24)
				<0.001*	<0.001*

Osteoporosis	2.5	4.8	1.1	2.17(1.08~4.36)	4.16
				0.03	(1.28~13.55)
					0.018
Rheumatoid arthritis	3.2	4.7	2.2	1.36(0.81~2.30)	2.02 (0.68~5.99)
				0.24	0.20
Rhinitis/ Hay fever	11.8	6.0	2.9	4.03(2.66~6.10)	1.92 (0.71~5.17)
				<0.001*	0.20
Stroke	1.5	0	0.5	3.23(1.11~9.39)	---
				0.03	
Thyroid disease	8.4	10.6	2.4	3.58(2.24~5.72)	4.64
				<0.001*	(1.97~10.90)
					<0.001*

* Statistically significant after Bonferroni correction for multiple testing i.e. the adjusted critical alpha level was taken as $p=0.003125$

Table 3.2 Logistic regression adjusted or non-adjusted for age, gender, body mass index and family membership

Medical diseases	Cases v. Controls		unaffected siblings v. Controls	
	OR(95% CI)		OR(95% CI)	
	P		P	
	unadjusted	adjusted	Unadjusted	adjusted
Asthma	3.89 (3.01-5.03)	4.41(3.11~6.25)	1.27 (0.92-1.76)	2.03
	<0.001*	<0.001*	0.15	(0.89~4.65)
				0.09
Epilepsy	6.12 (1.88-19.95)	3.90(1.21~12.65)	---	---
	0.003*	0.02		
Gastric ulcer	6.72 (3.50-12.89)	5.78(2.66~12.53)	2.30 (1.33-3.98)	4.74
	<0.001*	<0.001*	0.003*	(1.37~16.44)
				0.01
Hypercholesterolemia	2.22 (1.62-3.05)	2.79(1.84~4.25)	2.15 (1.61-2.87)	3.40
	<0.001*	<0.001*	<0.001*	(1.67~6.93)
				0.001*
Hypertension	2.33 (1.72-3.14)	2.38(1.62~3.51)	1.92 (1.43-2.57)	1.43
	<0.001*	<0.001*	<0.001*	(0.59~3.47)
				0.43
Myocardial infarction	2.67 (1.49-4.78)	3.12(1.47~6.63)	1.91 (1.13-3.23)	1.62
	0.001*	0.003*	0.015	(0.37~7.20)
				0.53
Osteoarthritis	2.94 (2.01-4.30)	3.22(1.95~5.34)	2.00 (1.41-2.84)	1.91
	<0.001*	<0.001*	<0.001*	(0.64~5.63)

				0.24
Rhinitis/ hay fever	4.03 (2.66-6.10)	5.54(3.02~10.15)	1.38 (0.85-2.26)	1.47
	<0.001*	<0.001*	0.20	(0.31~6.91)
				0.62
Thyroid disease	3.58 (2.24-5.72)	2.67(1.51~4.74)	2.15 (1.43-3.24)	3.64
	<0.001*	0.001*	<0.001*	(1.34~9.87)
				0.01

* statistically significant after Bonferroni correction for multiple testing i.e. the adjusted critical alpha level was taken as $p=0.003125$

Table 3.3 Familial Intra-trait correlation of physical diseases

Physical diseases	Tetrachoric rho (SE)	P
Hypercholesterolemia	0.34 (0.08)	<0.0001*
Hypertension	0.35 (0.07)	<0.0001*
Myocardial Infarction	0.32 (0.14)	0.03
Asthma	0.23 (0.06)	0.0004*
Hay fever	0.21 (0.09)	0.015
Diabetes Type1	-1.0	1.00
Diabetes Type2	0.36 (0.18)	0.06
Epilepsy	0.31 (0.23)	0.19
Gastric Ulcer	0.33 (0.10)	0.002*
Kidney Disease	0.18 (0.22)	0.34
Liver Disease	-1.0	1.0
Osteoarthritis	0.40 (0.08)	<0.0001*
Osteoporosis	0.52 (0.15)	0.0036
Rheumatoid Arthritis	0.10 (0.21)	0.47
Stroke	0.38 (0.23)	0.14
Thyroid Disease	0.25 (0.10)	0.015

* statistically significant after Bonferroni correction for multiple testing i.e. the critical alpha level was taken as $p=0.003125$

Table 3.4 Results from principal component analysis of the Varimax rotation with six factors

Item	component1	component2	component3	component4	component5	component6
Diabetes mellitus type2	0.57	-0.16	-0.03	-0.10	-0.06	-0.02
Myocardial infarction	0.59	0.18	0.02	-0.21	0.01	0.08
Hypercholesterolemia	0.63	0.16	-0.14	0.04	-0.10	-0.05
Hypertension	0.66	-0.17	0.08	0.14	0.08	-0.01
Osteoporosis	-0.04	0.76	-0.04	-0.08	-0.13	0.16
Thyroid disease	0.17	0.43	-0.23	0.03	0.21	-0.03
Gastric ulcer	-0.07	0.51	0.40	-0.02	-0.01	-0.06
Kidney disease	0.04	0.01	0.50	-0.03	0.32	0.26
Liver disease	-0.06	-0.03	0.73	0.06	0.10	0.03
Rheumatoid disease	0.16	0.02	0.46	-0.17	-0.34	-0.37
Allergic rhinitis	-0.03	-0.08	-0.02	0.87	-0.11	-0.09
Asthma	-0.01	0.14	0.14	0.37	-0.47	0.13
Osteoarthritis	0.18	0.30	0.07	0.39	0.22	-0.13
Diabetes Mellitus type 1	-0.01	-0.05	0.16	-0.08	0.73	-0.10
Stroke	0.29	-0.12	0.07	0.11	-0.08	0.48
Epilepsy	-0.05	0.17	0.02	-0.16	-0.10	0.76
Eigenvalue	2.07	1.21	1.18	1.11	1.05	1.01
% of variance explained	11.32	8.66	8.40	7.81	6.92	6.78
Correlation between siblings	0.24	0.19	0.07	0.13	0.02	0.05
	P<0.0001	P<0.0001	P=0.07	P=0.001	P=0.63	P=0.25

3.4 Discussion

3.4.1 Increased prevalence of physical diseases in depressed probands and siblings

Nine physical diseases were more common in patients with recurrent major depression than in psychiatrically healthy controls after controlling for family membership and adjusting for multiple testing. After further controlling for BMI, age and gender, eight physical diseases including asthma, gastric ulcer, hypercholesterolemia, hypertension, myocardial infarction, osteoarthritis, allergic rhinitis/hay fever and thyroid disease remained significantly more frequent in depressed patients. Similar prevalence rates of physical diseases were found in unaffected siblings of depressed patients, but only hypercholesterolemia showed a significant unaffected sibling/control group difference after multiple testing correction, which may be due to the small sample size in un-affected siblings. The overall pattern of results suggests a familial predisposition to physical diseases that is partially independent of a familial predisposition to depression. In our study, one quarter of depressed patients were obese, compared to only 10% of psychiatric healthy people, and obesity remain significantly associated with all of these eight diseases, except for gastric ulcer, in our models.

Our results largely support those reported by Farmer et al. (2008): asthma, gastric ulcer, hypertension, osteoarthritis, rhinitis/ hay fever and thyroid disease were found to be more frequent in patients with recurrent depression. We additionally found that two other conditions, namely hypercholesterolemia and myocardial infarction, were

also more common in cases. Notably, the two studies applied the same methods to assess psychiatric disorders and medical diseases. The only difference between the studies was the sample characteristics and source; the present study recruited sibling pairs with recurrent depression from the UK, Europe and the United States, whereas Farmer's study recruited unrelated patients with recurrent depression from the UK. Although in our study the prevalence rate of hypercholesterolemia (14.3% vs. 10.5%) and myocardial infarction (4.3% vs. 3.8%) seemed slightly higher in non-UK sites than in UK sites, this difference did not reach statistical significance after correction for multiple testing. All of the cases in the present study had recurrent depression, which is in keeping with recent findings (Gili et al., 2011) that medical conditions were more prevalent in patients with recurrent depression compared to those with a first episode.

3.4.2 Major depression and obesity

Our finding of an excess of obesity in depressed individuals is in keeping with some previous studies (Farmer et al., 2008, Simon et al., 2006), but not all (Papakostas et al., 2005, Patten et al., 2009). A recent meta-analysis (Luppino et al., 2010) found that depression had only a modest effect on the risk of obesity (OR=1.58). although some of included studies were community-based, in which the severity of depression was likely milder. Another study (Blaine, 2008) suggested that depressed patients are more likely to become obese at follow-up than non-depressed people, especially female adolescents. Long term use of antidepressant medication has an established association with weight gain, although the effect of individual antidepressants may vary (Serretti and Mandelli, 2010). Our cases had all experienced recurrent major

depressive episodes, and the majority have had multiple exposures to antidepressants, which may have contributed to the observed higher rates of obesity in our cases. A recent study in which the samples overlapped both with those of Farmer et al. (2008) and the recent study (Rivera et al., 2012) found that the association between the fat mass and obesity associated (*FTO*) gene and BMI was amplified in depressed patients compared to healthy controls, suggesting a first molecular-level clue as to why depressed patients may have an increased liability towards being overweight and developing diseases associated with being overweight.

3.4.3 Major depression and medical diseases

Many of the associations that we have found between common medical diseases and depression have been previously noted. Asthma has long been suggested to be associated with ‘psychosomatic’ factors, and the prevalence rate of depression in patients with asthma is higher than the general population (Afari et al., 2001). Corticosteroid use in patients with asthma could induce or exacerbate depression (Patten, 2000) and conversely, low mood may cause airway instability and asthma exacerbation (Ritz et al., 2001). Allergic diseases such as rhinitis/ hay fever have also been reported to be associated with major depressive episodes (Hurwitz and Morgenstern, 1999). A twin analysis suggested that 64% of the association between allergic diseases and depressive symptoms was due to shared familial vulnerability, mainly additive genetic factors (Wamboldt et al., 2000). Major depressive disorder, along with other mood or anxiety disorders, has been associated with peptic ulcer disease in a community sample (Goodwin et al., 2009). Hypertension is more prevalent in depressed patients (Adamis and Ball, 2000) and depression may be a risk factor for

the development of hypertension (Jonas et al., 1997). It has been postulated that the common underlying pathophysiology between hypertension and depression is related to increased sympathetic nervous system tone (Lambert et al., 2010). Osteoarthritis has also previously been linked to depression (Kim et al., 2011), and the intensity of perceived pain in patients with osteoarthritis is a predictor for depression (Hawker et al., 2011). Hypothyroidism has long been associated with depression (Teixeira et al., 2006), and high levels of thyroid stimulating hormone (TSH) have also been found to increase risk (Guimarães et al., 2009). Hypercholesterolemia has been linked with depression in the elderly (Tyrovolas et al., 2009) and the pathophysiology of mood disorder may relate to alternation of lipid profiles associated with high cholesterol (Papakostas et al., 2004). Depression has been shown to be associated with myocardial infarction, and to increase future risk (Yary et al., 2010). The findings of the present study build on previous reports, representing a systematic enquiry across a range of physical illnesses, and allowing us to examine possible familial aggregation of physical diseases in depressed siblings.

3.4.4 Familial aggregation and physical diseases

Our study of sibling pairs shed light on the familial risk of physical diseases comorbid with MDD. For example, we found a moderate correlation within sibling pairs for hypercholesterolemia, hypertension, gastric ulcer, osteoarthritis and asthma using tetrachoric correlation analysis, which is a technique to estimate the correlation between two theoretical normally distributed continuous latent variables underlying two dichotomous variables. Higher concordance rate of these diseases within pairs implies these diseases are familial in sibling pairs affected by depression. However,

we were unable to examine whether the familiarity of medical diseases was greater among depressed siblings compared to depressed patients and their unaffected siblings because of the small sample size of unaffected siblings.

The pattern of correlations between diseases reported here was in line with previous evidence. As might be expected, hypertension, hypercholesterolemia and myocardial infarction correlated with each other within individuals. A twin study (Scherrer et al., 2003) reported that there was a significant genetic correlation among depressive symptoms, heart disease and hypertension, which may explain this overlap, and our finding of increased prevalence of these diseases in depressed patients. A correlation within individuals was also found for allergic rhinitis and asthma. Allergic rhinitis often precedes the onset of asthma, and the association between asthma and rhinitis/hay fever is also likely to share a common immunopathological process (Braido et al., 2011). Other recent studies have also reported a high comorbidity between asthma and hay fever, with a phenotypic correlation between 0.58 and 0.65 (Fagnani et al., 2008, Willemsen et al., 2008). One previous study (Wamboldt et al., 2000) indicated that additive genetic factors account for a significant proportion of the covariance between atopy (the propensity to suffer from allergies) and depressive symptoms. In addition, allergic respiratory disease has been shown to be a risk factor for hypertension (Aung et al., 2010), and patients with asthma were 1.3 times more likely to have hypertension than patients without asthma (Dogra et al., 2007). Previous studies have shown that osteoarthritis is associated with several diseases such as gastric ulcer, hypercholesterolemia, hypertension and osteoporosis. Although we do not have accurate records of medication use in our cases, patients with osteoarthritis are likely take more non-steroid anti-inflammatory drugs (NSAID),

which may subsequently contribute to gastric ulcer (Geis et al., 1991). Chronic pain is a core symptom of osteoarthritis. Patients with chronic pain tend to report more physical conditions and most of self-reported chronic physical conditions are significantly associated with chronic pain (Dominick et al., 2012). This might explain why osteoarthritis showed associations with a number of other medical diseases.

Most complex diseases are thought to involve both genetic and environmental factors. For example, lipid profile abnormalities are as a risk factor for cardiovascular disease, and the level of total cholesterol aggregates within families (de Miranda Chagas et al., 2011). It has been shown that heritable factors account for more than 50% of the population variation for serum total cholesterol (Fuentes et al., 2000b). Blood pressure is an important factor for various cardiovascular diseases, and also aggregates within families (Fuentes et al., 2000a); with the genetic contribution to blood pressure estimated at between 30% and 60% across ethnicities (Kupper et al., 2005, Snieder et al., 2003). Estimates of the heritability of osteoarthritis range from 39% to 60%, depending on the location and definition of osteoarthritis (symptomatic or radiographic findings). It is unclear whether the variation in estimates of heritability could be attributed to effects on different joints, or is due to a heterogeneous phenotype definition (Riyazi et al., 2005). Asthma and hay fever have been linked to elevated levels of serum Immunoglobulin E (IgE), and the prevalence rate of asthma has increased in recent years (Thomsen et al., 2011). Familial aggregation of asthma has been widely reported, and estimates of heritability range from 60% - 91%, depending on the definition of asthma (Thomsen et al., 2011, Willemsen et al., 2008). Furthermore, the genetic correlation between asthma and

hay fever has been estimated to be 0.58, and the phenotypic correlation between the two diseases appears to be entirely genetically mediated (Fagnani et al., 2008).

3.4.5 Potential mechanism linking depression and medical diseases

As noted in Chapter 1, there are various physiological mechanisms that could mediate the relationship between physical illnesses and depression. One possible overlapping mechanism is hypothalamus-pituitary-adrenal (HPA) axis dysfunction, with elevated cortisol levels reported in both depressed and obese people.

Heightened levels of cortisol have shown to contribute to weight gain, with a particular impact on increased visceral adiposity (Pasquali et al., 2002). The results of the present study, which are in line with previous reports of increased obesity and cardiovascular diseases in depressed patients vs. controls motivate further studies investigating whether HPA axis dysfunction, potentially related to chronic stress, could mediate this relationship.

Another potential mechanism for the relationship is chronic low-grade inflammation. Physical illnesses and depression are both related to raised levels of inflammatory mediators. As discussed in Chapter 1, pro-inflammatory cytokines such as tumor necrosis factor- α (TNF- α), interleukin-1 (IL-1), IL-6 and interferon- γ have been reported to be elevated in depression (Kenis and Maes, 2002). In addition, elevated levels are reported in gastric ulcer (Sugimoto et al., 2010), asthma (Woodruff et al., 2009), and rhinitis and osteoarthritis (Valdes and Spector, 2010). An additional inflammatory marker, high sensitivity C-reactive protein (hsCRP) has also been linked to depression (Kling et al., 2007), and past-year major depression is a risk

factor for subsequent ischemic heart disease (Surtees et al., 2008). The present report of increased rates of physical diseases related to inflammatory processes in depressed patients motivates further studies examining whether inflammation could mediate this link.

3.4.6 Limitations

Some limitations of our study should be taken into account when interpreting the results. First, history of medical diseases and BMI was collected via self-report, rather than from a review of medical case records. However, in our previous study (Farmer et al., 2008), 93% of self-reported physical illnesses were confirmed by patients' General Practitioners (GPs), and the agreement for reporting on each disease between GPs and self-report ranged from 0.65 to 0.91 (kappa co-efficient), suggesting substantial to very good agreement.

A second limitation is that this study used a cross-sectional study design. Such a design can only show the association between depression and physical illness, and cannot determine the temporal relationship. Third, patients with recurrent depression who retrospectively report their physical illnesses may be biased by their present mood state, although depressive symptoms measured using the Beck Depression Inventory only accounted for 1.5% of the variance in the number of physical illnesses in a previous study (Farmer et al., 2008). Fourth, depressed people may be more likely to be referred or investigated for physical illness compared to non-depressed people (Berkson, 1946). Fifth, familial aggregation of physical illness does

not necessarily reflect co-segregation with depression, since we did not have enough non-affected family members in the sample to investigate this.

A further limitation was that our study sample consisted of siblings. This meant that it was not possible to differentiate genetic factors from shared environmental factors – we were only able to estimate the ‘familiality’ of the disorders. Until recently, differentiation of genetic and shared environmental factors could only be achieved using twin studies, but a recent method called genome wide complex trait analysis (Yang et al., 2011) provides a method of partitioning these two sources of variance using genome wide genetic marker data.

Despite these limitations, our study provides compelling evidence that certain physical diseases and traits including obesity are more prevalent in depressed patients. Familial aggregation of physical diseases, and cross-trait correlations further suggest the importance of comorbid physical diseases in depressed patients. These findings suggest that clinicians should be alert to the presence physical diseases in depressed patients. Future studies should focus on finding the common pathways linking depression and physical diseases.

Chapter Four

Neuroticism is associated with asthma and total number of physical disease but no familial correlation exists between neuroticism and asthma

Abstract

In Chapter 3, evidence showing that major depressive disorder (MDD) is associated with various physical diseases was presented. Previous studies have suggested that there is an association between MDD and certain personality traits, especially neuroticism, so it is possible that the association between MDD and physical diseases could also be related to personality traits that render individuals susceptible to the effects of stress. After controlling for age, sex, body mass index (BMI) and depression status, neuroticism was positively associated with the *total number* of physical diseases, although the effect was small. A similar pattern was found for the presence of *any* physical disease. Of the specific diseases investigated, only asthma was modestly but significantly associated with neuroticism, after correcting for multiple testing. Since both asthma and neuroticism were correlated in siblings suggesting familiarity, the familial correlation between neuroticism and asthma was then examined. However, the bivariate modelling results did not find evidence for a significant familial correlation between the phenotypes. As such, it is therefore unlikely that neuroticism plays an important role in the association between MDD

and physical diseases. Even in the case of asthma where a modest phenotypic correlation was observed, there was no evidence to suggest that common genetic factors contribute to both neuroticism and asthma.

4.1 Introduction

In the previous chapter, evidence showing that several common physical diseases were significantly more common in patients with recurrent depression than in controls was presented. In addition, there was evidence for familiarity of physical diseases in depressed patients: siblings who were both affected with depression tended to be concordant for coexistent physical diseases. There was also an increased rate of physical disorders among unaffected siblings compared with controls, although only hypercholesterolemia showed a significant unaffected sibling/control group difference after multiple testing correction, potentially due to the small sample size. In this chapter, I explore whether personality traits play a role in the comorbidity between recurrent depression and physical disorders, first by examining whether personality factors, as measured by the Eysenck Personality Questionnaire (EPQ) (Eysenck and Eysenck, 1975) are associated with physical disorders, and second by examining whether any personality traits phenotypically associated with a physical disorder show evidence of familial overlap with the physical disorder, using structural equation modelling on sib-pair data.

The hypothesis that personality might be involved in the relationship between physical diseases in depression is evidenced by previous studies that have shown a relationship between depression and personality traits, both in terms of susceptibility to depression (Farmer et al., 2002) and its prognosis (Ranjith et al., 2005). In addition, studies have shown that certain personality traits and disorders are associated with various somatic complaints or physical diseases. In particular, personality disorders,

which represent the extremes of various personality traits, might be expected to predict physical complaints, fatigue and pain (Powers and Oltmanns, 2012). In a recent study, borderline personality disorder was associated with an increased risk of numerous physical diseases such as cardiovascular disease and arthritis (El-Gabalawy et al., 2010). In addition, individuals with symptoms of personality disorder were reportedly more likely to develop coronary artery disease (Pietrzak et al., 2007). There is also evidence to suggest that people with extreme scores on certain personality traits have more somatic complaints or physical diseases. For example, studies have shown that a high levels of neuroticism are associated with the total number of somatic symptoms (Costa and McCrae, 1985, Charles et al., 2008). Neuroticism has been reported to be significantly more strongly related to psychosomatic symptoms than to infectious/ allergic symptoms (Rosmalen et al., 2007). Neuroticism has also been reported to be associated with ulcer, asthma and heart disease (Friedman and Booth-Kewley, 1987).

Studies have consistently reported a strong association between major depression and neuroticism, although there is evidence that neuroticism as a *trait* is partly confounded with depressive *states* (Farmer et al 2002). However, the question of whether the association between depression and physical diseases (Farmer et al., 2008) is mediated by neuroticism warrants investigation, and could have implications for understanding the relationship between the two.

Although most studies focus on neuroticism, other personality traits have also been reported to be associated with physical diseases. For example, conscientiousness is associated with significantly reduced likelihood of numerous physical diseases

(Goodwin and Friedman, 2006). Extraversion has been reported to relate to positive health outcomes for both current and retrospective symptoms (Williams et al., 2004). A recent study (Klabbers et al., 2013) suggested that a comprehensive psychosocial profile including psychological attributes, psychological functioning and coping style could predict health risk more accurately than traditional single personality traits; and an adverse psychosocial profile could also indicate increased risk of mortality and poorer self-rated health.

In summary, in this chapter will first examine the relationship between personality traits and the number of reported physical diseases/ the presence of any physical disease. Phenotypic associations will then be investigated using bivariate modeling to explore whether there is familial overlap between specific physical diseases or physical diseases generally and personality traits.

4.2 Methods

The analytic methods used in this study are described in detail in Chapter 2. The study sample was identical to that in chapter 3.

For simplicity in the analysis, each physical disease (event) was treated as independent although in practice, the findings in Chapter 3 indicate that some physical diseases tend to cluster together.

4.3 Results

Descriptive statistics indicated that traits of neuroticism and psychoticism were significantly higher in depressed people than healthy controls (mean (SD), neuroticism: 15.45 (5.06) v 5.26 (3.30), $P<0.001$; psychoticism: 3.81 (2.22) v 2.92 (1.79), $P<0.001$). In contrast, extraversion was significantly higher in healthy controls than depressed subjects (10.13 (5.16) v 14.78 (4.68), $P<0.001$) (see Table 4.1). The mean of the total number of physical diseases was 0.90, and the variance 1.52 across the whole sample. Neuroticism was significantly positively associated with the number of physical diseases (Coef.=0.04, SE=0.005, $P<0.001$), whilst extraversion (Coef.=−0.02, SE=0.005, $P=0.001$) and psychoticism (Coef.=−0.03, SE=0.01, $P=0.02$) were negatively associated with the number of physical diseases reported on. The effect sizes of these associations were small, and only neuroticism was still associated with the number of physical diseases after taking into account depressive status, sex, age and body mass index (BMI) (Coef.=0.02, SE=0.007, $P=0.004$). The results were similar when the dependent variable was changed to the presence of any physical disease. Only neuroticism was associated with the presence of any physical disease after controlling the confounding factors (as above) and adjusting for multiple testing (Coef.=0.05, SE=0.01, $P=0.001$).

Given that neuroticism was associated with the presence of any physical disease, we then examined whether specific physical diseases were associated with neuroticism. Among 16 common physical diseases, only asthma (Coef.=0.07, SE=0.02, $P<0.001$) was associated with neuroticism after controlling for confounding factors and multiple testing (Table 4-2).

Table 4.1 Personality traits difference between individuals with MDD and healthy controls

		Total	Cases	Controls	p-value
Neuroticism	Mean	11.80	15.45	5.26	<0.001
	(SD)		(5.06)	(3.30)	
	Median	12.00	16.00	5.00	
Extraversion	Mean	11.90	10.13	14.78	<0.001
	(SD)		(5.16)	(4.68)	
	median	12.00	10.00	15.00	
Psychoticism	Mean	3.51	3.81	2.92	<0.001
	(SD)		(2.22)	(1.79)	
	median	3.00	3.00	2.00	

Table 4-2 Mixed-effect logistic regression controlling for age, gender, body mass index, extraversion, psychoticism and depression status

	Coef. (95% CI)	SE	P
Asthma	0.07 (0.04~0.10)	0.02	<0.001*
Type 1 Diabetes	-0.06 (-0.18~0.07)	0.06	0.37
Type 2 Diabetes	0.05 (-0.06~0.15)	0.05	0.41
Epilepsy	-0.02 (-0.12~0.07)	0.05	0.65
Gastric Ulcer	0.09 (0.03~0.15)	0.03	0.004
Hypercholesterolemia	-0.02 (-0.07~0.03)	0.02	0.39
Hypertension	0.01 (-0.03~0.05)	0.02	0.67
Kidney Disease	0.01 (-0.07~0.09)	0.04	0.71
Liver Disease	0.03 (-0.05~0.12)	0.04	0.43
Myocardial Infarction	0.04 (-0.02~0.11)	0.04	0.43
Osteoarthritis	0.07 (0.02~0.13)	0.03	0.01
Osteoporosis	0.01 (-0.08~0.10)	0.05	0.79
Rheumatoid Arthritis	0.00 (-0.07~0.07)	0.04	0.98
Allergic Rhinitis	0.02 (-0.02~0.06)	0.02	0.33
Stroke	0.04 (-0.08~0.15)	0.06	0.53
Thyroid Disease	-0.01 (-0.06~0.04)	0.03	0.65

Although the size of the association between asthma and neuroticism was small, data from sib-pairs both affected by depression was used to investigate whether there was a familial correlation between asthma and neuroticism (Figure 4.1). The model described in Wood, Rijdsdijk et al. (2011) was used, with fixed parameters for the selection trait (i.e. disease status). The threshold for asthma status was fixed to a z-value of 1.64 to give a population prevalence of 10% (Brogger et al., 2003). Additional parameters were fixed to the expected population estimates based on previous reports, with the familiarity of asthma fixed at 60% (Thomsen et al., 2010). After removing the parameter for the correlation between asthma and neuroticism, the reduced model was not inferior in terms of model fit to the full model (difference chi-squared=1.48, difference d.f.=1, $P=0.22$), suggesting that there is no familial correlation between asthma and neuroticism.

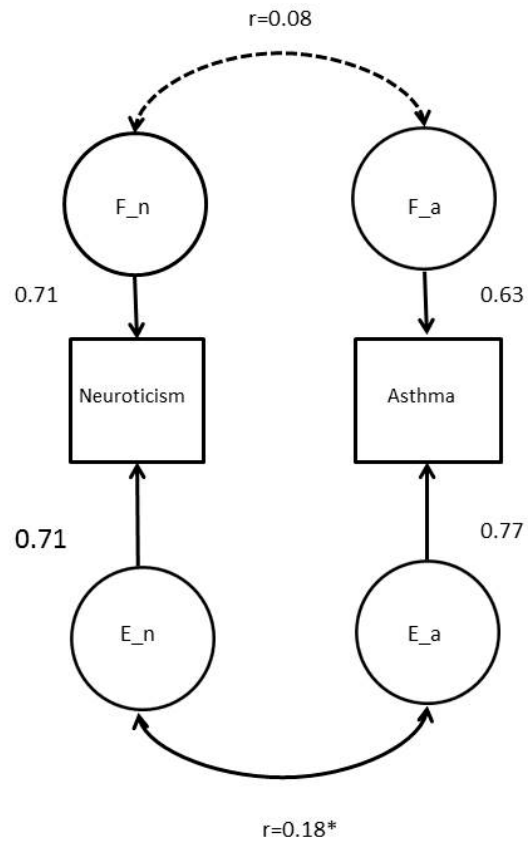


Fig 4-1 Parameters F_n , F_a , E_n , E_a are estimates from the Cholesky model, partitioning the variance of the overlap between neuroticism and asthma into familial and non-shared environmental factors. Significant paths are indicated with an asterisk.

4.3 Discussion

These results indicate that neuroticism is associated with the reported number of physical diseases, as well as with the presence of any physical diseases in the study sample. Several previous studies have reported similar results, indicating that neuroticism is associated with total number of physical symptoms or physical diseases after controlling comorbid psychiatric disorders (Neeleman et al., 2004, Goodwin and Friedman, 2006, Williams et al., 2004, Johnson, 2003, Rosmalen et al., 2007). As noted in the introduction, one study argued that neuroticism was more associated with psychosomatic symptoms than with infectious/ allergic symptoms (Rosmalen et al., 2007), and a second suggested that neuroticism is only related to ‘tension’ type diseases such as hypertension, migraine or neck pain (Johnson, 2003). However other studies have suggested that neuroticism has more general effects, and is related to poorer health behaviour self-efficacy, especially in the presence of low extraversion (Williams et al., 2004). This pattern is in contrast with conscientiousness, which was reported to be associated with lower rates of physical diseases (Goodwin and Friedman, 2006).

Several mechanisms might explain the association between neuroticism and increased physical illnesses. First, neurotic people are more likely to over-report their physical problems, or be more sensitive to or anxious about their bodily sensations and visit their doctors more frequently. Therefore, more physical diseases may have been diagnosed by their doctors. The absence of a link between neuroticism and premature natural mortality supports the possibility of over-reporting of physical

illnesses as an explanation for the association we have found (Neeleman et al., 1998). However, other studies have argued that neurotic people seek medical help during early stages of physical diseases and take interventions earlier, resulting in a lower mortality rate (e.g. O'Carroll et al., 2001).

Second, the association between neuroticism and physical conditions could be a reverse causal relationship, i.e. people with more physical conditions tend to be more neurotic as a result. For example, Neeleman et al. (2004) reported that neuroticism was associated not only with current somatic morbidity, but also with future morbidity during a follow-up period. On the other hand, somatic morbidity was also associated with increased neuroticism during follow-up.

An alternative explanation for the link between neuroticism and physical conditions is via psycho-physiological pathways. Previous studies have shown that certain specific personality traits are associated with increased levels of interleukin-6 (IL-6) and C-reactive protein (CRP) in clinically depressed individuals, and in the general population (Coccaro, 2006, Marsland et al., 2008). A recent study (Armon et al., 2013) found further evidence for positive associations between neuroticism and extraversion and two inflammatory markers at baseline and when measuring change over time across a four year follow-up period. Although the associations were significantly positive, only a small proportion of variance (approximately 2%) of these two inflammatory markers was explained by the personality traits. Turiano et al. (2013) reported a significant interaction effect between neuroticism and conscientiousness on IL-6 level, suggesting that people with both high neuroticism and high conscientiousness had lower IL-6 plasma levels (indicative of reduced

inflammation) compared to people with all other configurations of neuroticism and conscientiousness. However, this study did not investigate the relationship between neuroticism and specific physical diseases.

In general, our findings are in keeping with previous studies reporting a positive relationship between neuroticism and self-reports of illnesses. However, the effect was weak overall, as reflected in the small correlation coefficients. In the analysis of single diseases, only asthma was still significantly associated with neuroticism after controlling for potential confounding factors (Coef.=0.07, SE=0.02, $P<0.001$). This finding is in keeping with previous studies suggesting a link between asthma and various stress and psychological factors. A large scale community study (Huovinen et al., 2001) suggested that asthma prevalence was associated with high levels of neuroticism. A further study reported that high neuroticism predisposed to develop asthma (Loerbroks et al., 2009). Another study (Wainwright et al., 2007) suggested various psychological factors, such as current mood disorder, adverse childhood circumstances, stressful life events and poor social support were associated with asthma hospital admission.

In our preliminary analysis, asthma was the only disease that was still related to neuroticism after controlling for potential confounding factors. We then examined the familial relationship between asthma and neuroticism, and found no significant correlation between asthma and neuroticism within our sample of depressed siblings. As such, this analysis did not support a common familial aetiology hypothesis for the co-occurrence of asthma and neuroticism. To the best of our knowledge, no previous

studies have investigated the genetic or familial correlation between neuroticism and asthma. One potential criticism of the approach I have taken is that sometimes fitting a Cholesky is invalid when it does not produce fit statistics that are distributed as a χ^2 or if the distribution of the fit statistic is χ^2 , then the degrees of freedom are not always the difference between the number of parameters in the general model less the number of parameters in a constrained model (Carey, 2005). Using either general model or constrained model does not change the statistical significance. However, the familial relationship between neuroticism and other physical conditions included in the present study has been explored in previous research. One study (Charles et al., 2008) used co-twin control analysis, and reported that genetic influences mediate the association between neuroticism and four of the thirteen physical conditions included in the present study. The results showed that familial influence explained the association between neuroticism and following diseases: joint pain, tension headache, migraine and coronary heart disease. Notably, asthma was not included in that analysis. Another recent twin study (Vassend et al., 2012) examined the heritability and genetic correlation between neuroticism and somatic complaints. The heritability of neuroticism was 46%, and of somatic complaints was approximately 45%. Nearly 60% of the phenotype correlation between neuroticism and somatic complaints could be attributed to common genetic factors. A key difference between their study and ours was the methods used to assess physical health. Whereas Vassend et al., (2012) used subjective reports of somatic symptoms, the present study used self-report of clinician diagnosed asthma.

Several limitations of this study should be taken into account when interpreting these results. First, physical diseases were measured using self-report of what the subjects

had been told by their doctors, rather than objective clinical diagnoses obtained from medical records. This raises concerns about possible recall bias or over-reporting. However, a previous sub-group analysis of one of the studies contained in this study (DeCC) demonstrated that self-reported physical diseases are reliable in relation to GP records (Farmer et al., 2008). A second limitation of the study was the fact that it was a cross-sectional in design. As such, temporal analysis could not be used to explore whether level of neuroticism predicts change in asthma symptoms over time. A third limitation is that, although a sibling design is able to examine shared familial effects, it cannot separate genetic from shared environmental effects. A fourth limitation is that confidence intervals could not be computed using the Mx package, because the data were ordinalised. Fifth and finally, the analysis was restricted to sib-pairs where both were affected by recurrent depression. As such, this sample was likely selected from the high end of the distribution of neuroticism, and restricted variance may have attenuated the effect sizes of the associations between personality traits and physical diseases.

In summary, these findings suggest that neuroticism was associated with both the total number of physical diseases, and the presence of any physical diseases, after controlling for potential confounding factors. When the relationship between neuroticism and individual common chronic physical diseases was investigated, only asthma was significantly associated with neuroticism after correction for multiple testing. No familial correlation among sib-pairs was observed between neuroticism and asthma. However, this issue may warrant further investigation in family or twin data that are more representative of the population as a whole with respect of the distribution of neuroticism.

Chapter Five

Estimation of the genetic correlation between major depressive disorder and body mass index using common genetic variants

Abstract

As described in Chapter 1, both obesity and major depressive disorder (MDD) are prevalent in developed countries and cause a huge disease burden. Previous studies, including Chapter 3 of this thesis, have reported an association between obesity and MDD, but it is unclear whether common genetic factors are responsible for the overlap between these two disorders. A recently developed method was used to estimate the proportion of variance in disease liability explained by common single nucleotide polymorphisms (SNPs), and the genetic correlation between obesity and MDD in 3872 unrelated individuals from the RADIANT study, plus an additional 1645 individuals from the Munich depression study. Findings indicate that the percentage of the overall variance accounted for by common SNPs is 15% for BMI, and 32% for MDD. Our results support evidence suggesting that both BMI and MDD are heritable, with a significant proportion of phenotypic variance explained by the additive genetic effects of common SNPs. Furthermore, this is the first study to suggest that there is a significant large, but as yet imprecise, genetic correlation between BMI and MDD.

5.1 Introduction

The prevalence of obesity has been reported to be approximately 30% in developed countries (Flegal et al., 2010) while the life time prevalence rate of major depressive disorder (MDD) may be as high as 15% to 20% (Blazer and Kessler, 1994). Both obesity and MDD are associated with various chronic medical diseases and have major impacts on public health (Sturm, 2002, Katon, 2003). As discussed in Chapter 1, there have now been several studies indicating a strong association between MDD and obesity (Luppino et al., 2010, Farmer et al., 2008, Rivera et al., 2011). However, the reasons as to why they should cluster together remain unclear.

5.1.1 Potential underlying mechanisms contributing to obesity and MDD

Chapter 1 addressed a range of possible factors that could explain the association, including psychological factors such as lack of confidence and stigma, especially in obese women (Puhl and Brownell, 2003), sedentary life style, overeating and chronic antidepressant use (Cassidy et al., 2004). Chapter 1 also considered that both depression and obesity could be influenced by a common underlying pathophysiological mechanisms such as low grade inflammation and hypothalamic-pituitary-adrenal axis dysfunction (Dantzer et al., 2008), and that both obesity and MDD may share some genetic variance (Afari et al., 2010).

Previous work has proposed a moderator-mediator framework has also been proposed to explain the relationship between obesity and depression (Faith et al.,

2002). Potential moderators included socio-demographic factors, predisposing genotypes and binge eating disorder, while negative verbal commentary, eating and physical activities were suggested to be potential mediating factors.

5.1.2 Twin studies suggested high heritability in obesity and MDD but genome-wide association studies have limited findings

As noted in Chapter 1, family and twin studies have indicated that both BMI and MDD have a substantial heritable component ($> .4$), with variation related to whether participants were recruited from community or clinical settings (Sullivan et al., 2000, McGuffin et al., 1996). Given importance of genetic factors in contributing to population variance on both traits, the issue of whether common genetic factors may contribute to the clustering of MDD and obesity warrants investigation.

Despite the evidence for a substantial heritable component for both BMI and MDD, the results of genome-wide association studies (GWAS) have been somewhat disappointing. As discussed in Chapter 1, GWAS have identified at least 32 common variants contributing to BMI, but together they only account for less than 2% of variance (Speliotes et al., 2010). Furthermore, no single nucleotide polymorphism (SNP) passing genome-wide significance threshold in MDD GWAS has yet been replicated in an independent sample (Wray et al., 2010). These findings have raised concerns about ‘missing heritability’ (Manolio et al., 2009), that is the gap between

the estimates of heritability from twin studies and the total variance actually explained by SNP associations.

5.1.3 Genome-wide complex trait analysis provides different method to investigate “SNP heritability”

The heritability estimates derived from twin methods rely on several major assumptions such as equal environment assumption (Hettema et al., 1995), which might not be sound. Recently, an alternative method has been developed to estimate the heritability of non-Mendelian traits, called genome-wide complex trait analysis (GCTA) (Yang et al., 2011a). GCTA correlates genomic similarity across hundreds of thousands SNPs derived from GWAS data with phenotypic similarity pair by pair across large samples of unrelated individuals. GCTA decomposes the phenotypic variance into additive genetic (“SNP heritability”) and non-genetic components of variance, and has been successfully applied to various traits such as height, weight, and a variety of psychiatric and medical diseases (Yang et al., 2011a, Lee et al., 2011, Yang et al., 2011b, Lee et al., 2012b). The genetic influence on a trait or disease estimated by GCTA is usually smaller than the heritability found in twin studies. One reason for this is that GCTA only includes common variants tagged by GWAS which are in imperfect linkage disequilibrium with causal SNPs. Recently, the GCTA method has been extended to perform bivariate analysis of two quantitative traits in general populations, or two qualitative traits in case-control studies. by decomposing the phenotypic covariance between traits into components of covariance (Lee et al., 2012a). In addition, GCTA can run bivariate analysis using both binary/ continuous

traits, or a binary trait and a continuous trait. In this study, we used GCTA to investigate the phenotypic variance of BMI and MDD explained by genetic variants captured by GWAS data, and the genetic correlation between BMI and MDD related to these variants.

5.2 Results

After quality control procedures, the sample consisted of 3872 individuals, including 2865 cases and 1007 controls from the RADIANT studies. These data were used to estimate the genetic relationship matrix using autosomal SNPs that also passed stringent quality control procedures. The reliability of self-reported height and weight measurements were checked against measured height and weight in the GENDEP dataset, where both types of measure were available. The correlation for measured versus self-reported height was 0.95 ($P<0.0001$) and for measured versus self-reported weight 0.97 ($P<0.0001$). The correlation for BMI-based measured data versus self-report was 0.95 ($P<0.0001$).

Analyses were initially performed on the RADIANT sample ($N=3872$). The proportion of phenotypic variance in BMI and MDD related to the additive effects of common SNPs was estimated at approximately 0.17 (S.E.=0.12, $P=0.10$) and 0.36 (S.E.=0.12, $P=0.001$), respectively. The residual variance for BMI was 22.04 (S.E.=3.48), and for MDD, 0.09 (S.E.=0.02). The residual covariance between BMI and MDD was -0.09 (S.E.=0.18). The estimated genetic correlation between BMI and MDD was 0.61 (SE=0.26, $P=0.02$). To obtain greater power, an additional 1645 individuals ($N=793$ cases, $N=852$ controls) genotyped with the same commercial chip were added to the sample. The results of analysis on the combined sample suggested that common SNPs explained 0.15 (SE=0.09, $P=0.04$) of variance in BMI and 0.33 (SE=0.08, $P=5*10^{-6}$) of variance in MDD. However, the estimated genetic correlation between BMI and MDD was no longer significantly different from zero using the conventional 5% alpha level ($rG=0.40$, SE=0.23, $P=0.08$).

5.3 Discussion

Results conducted incorporating both the RADIANT and Munich-GSK data indicate that the “SNP heritability”, or proportion of variance explained by additive genetic effects, was 0.15 for BMI and 0.33 for MDD. As expected, the genetic variance explained by common variants estimated using GCTA was lower than the heritability estimated from twin studies. The reasons are probably two-fold. First, GCTA only estimates the effect of common variants, rather than all genetic variants including rare alleles that might contribute to variation. Second, there is incomplete linkage disequilibrium between casual variants and genotyped variants, meaning that the effect will be exaggerated if un-genotyped real causal variants have a lower allele frequency than the tagged SNPs in commercial arrays (Yang et al., 2010). That said, our results confirm that both BMI and MDD are heritable, and further suggest that a significant proportion of phenotypic variance can be explained by the additive genetic effects of common SNPs. In addition, our findings are largely in line with previous univariate studies, which have suggested that the “SNP heritability” of BMI is 17% (Yang et al., 2011b) and 32% for MDD (Lubke et al., 2012). Although GWAS have identified dozens of genetic risk alleles for BMI, the cumulative effects of these variants only explain a tiny proportion of the phenotypic variants. In MDD, no genome-wide significant SNPs have replicated. Our results suggest that the ‘missing heritabilities’ of BMI and MDD are not so much missing as hiding. Further variants exerting very small effects could be identified by increasing the sample size in GWAS studies to obtain enough power. The different magnitudes in the proportions of additive genetic variance captured by common variants between BMI

(18%~33%) and MDD (44%~83%) could suggest that the allelic architecture of these two complex traits differs.

When calculating the genetic correlation between a continuous trait and a binary trait, GCTA analysis treats both traits as though they are continuous. This does not influence the estimate of the genetic correlation, as this estimate is not affected by scale or ascertainment (Lee et al., 2012a). However, GCTA will not treat the binary trait as a disease, and therefore the estimate of the variance explained for the disease trait is not adjusted for scale and ascertainment (i.e., it is not on the scale of liability). Therefore, the results for the disease trait might be slightly different from those on using a univariate analysis on that trait or a bivariate analysis with a continuous trait.

The GCTA genetic correlation between BMI and MDD was 0.61 in the RADIANT sample, and 0.40 in the combined sample, with the 95% confidence interval for the latter estimate overlapping with zero, meaning that it was not significant using conventional criteria. As such, conclusions should be drawn with caution. The discrepancy between these two estimates and the large standard errors suggest that bivariate GCTA analysis almost certainly needs even larger sample sizes to arrive at definitive estimates. Although the phenotypic variance explained by genetic factors estimated using GCTA is usually smaller than that reported in twin studies, the genetic correlation between BMI and MDD should be independent of the estimate of heritability, and therefore unbiased. This is because the ratio between genetic variance and genetic covariance are assumed to be biased to the same extent in both

traits, so they offset each other. In addition, the genetic correlation between two traits can be high even when the heritability of both individual traits is low.

Family and twin studies have previously suggested a significant genetic correlation between BMI and depression, but the findings have been somewhat inconsistent. A recent large family-based study (Choy et al., 2009) in a genetically isolated community attempted to disentangle the contribution of shared environment factors and genetic factors to the correlation between depressive symptoms and body composition (including BMI, lean mass index, fat mass index and waist-hip ratio). Although there was a significant phenotypic correlation between depressive symptoms and body composition, there was no evidence to support the contribution of shared genetic factors to the co-occurrence between depressive symptoms and body composition. A twin study in males (McCaffery et al., 2003) reported an association between obesity-related metabolic syndrome and depressive symptoms, but this appeared to be attributable to common environmental factors rather than to shared genetic factors. In contrast, twin study in females (Afari et al., 2010) suggested that 12% of the genetic component of depression was shared between depression and obesity. Our results suggest that an even larger genetic correlation could exist, but a much larger sample is needed to increase the reliability of the estimate.

One advantage of our study is the robustness of MDD diagnoses. In all previous studies examining a possible genetic relationship with obesity, depression was assessed using self-reported scales of symptoms, or self-report of doctor-diagnosis of

depression. For our subjects, the diagnosis was based on face-to-face semi-structured interview, and at least moderate severity was required for inclusion. This is likely to be of relevance, since there is evidence that the phenotypic association between obesity and depressive disorder is stronger than that between obesity and depressive symptoms (Luppino et al., 2010). This may explain a stronger genetic correlation between clinical depression and BMI than between BMI and self-reported depressive symptoms.

5.4 Limitations

One potential limitation of this study is that the calculation of BMI was based on self-reported weight and height. However, for a substantial subset of subjects ($N = 811$), we were able to compare self-reported weight and height with measured weight and height, and we found that the correlations were very high. This suggests that using a self-report of BMI does not introduce much error, and contradicts the claim (Gorber et al., 2007) that people systematically under-report their BMI. An additional potential problem is that this analysis combined studies where the subjects were collected from multiple sites. Although they were all of white European origin, there is still a risk of introducing population genetic substructure, as well as adding country specific environmental variation (non-genetic risk factors). Both effects could bias the estimate of heritability. To mitigate this problem, principal components derived to estimate population substructure were included as fixed effects, although this approach cannot be regarded as foolproof (Browning and Browning, 2011). A final limitation is that, although RADIANT is one of the largest

samples on which both information on clinical depression and BMI is available, and although we expanded the dataset by adding in the Munich sample, we are still faced with an imprecise estimate of the true size of the genetic correlation between BMI and depression. Much larger sample sizes would be desirable for future studies.

5.5 Conclusion

The present study indicated that fairly substantial proportion of the variance of BMI and MDD can be explained by common SNPs tagged by commercial arrays, although estimates are lower than the heritability suggested by twin studies. It is also the first to demonstrate using GCTA that there may be a genetic correlation between BMI and MDD. However, the results at this stage are only suggestive, and further studies with even larger sample sizes are needed.

Chapter Six

A genetic risk score combining 32 SNPs is associated with body mass index and improves obesity prediction in people with major depressive disorder

(This chapter forms the basis of a paper of the same title by Hung CF et al; that has now been published in BMC Medicine 13:86, 2015)

Abstract

Obesity is strongly associated with major depressive disorder (MDD) and various medical diseases. As described previously, genome-wide association studies (GWAS) have identified multiple risk loci robustly associated with body mass index (BMI). In this study, we aimed to investigate whether a genetic risk score (GRS) combining multiple BMI risk loci might have utility in the prediction of obesity in patients with MDD. Linear and logistic regression models were conducted to predict BMI and obesity, respectively, in three independent large case-control studies of major depression (RADIANT, GSK-Munich, PsyCoLaus). The analyses were first performed in the whole sample and then separately in depressed cases and controls. An unweighted GRS was calculated by summation of the number of risk alleles. A weighted GRS was calculated as the sum of risk alleles at each locus multiplied by

their effect sizes. Receiver operating characteristic (ROC) analysis was used to compare the discriminatory ability of predictors of obesity.

In the discovery phase, a total of 2,521 participants (1,895 depressed patients and 626 controls) were included from the RADIANT study. Both unweighted and weighted GRS were highly associated with BMI ($p < 0.001$) but explained only a modest amount of variance. Adding ‘traditional’ risk factors to GRS improved the predictive ability significantly, with the area under the curve (AUC) in the ROC analysis increasing from 0.58 to 0.66 (CI 0.62-0.68, $\chi^2 = 27.68$, $p < 0.0001$). Although there was no formal evidence of interaction between depression status and GRS, there was further improvement in AUC in the ROC analysis when depression status was added to the model (AUC=0.71, CI 0.68-0.73, $\chi^2 = 28.64$, $p < 0.0001$). We further found that the GRS accounted for more variance of BMI in depressed patients than in healthy controls, and discriminated obesity better in patients than in controls. We later replicated these analyses in two independent samples (GSK-Munich and PsyCoLaus) and found similar results.

As such, the GRS proved to be a highly significant predictor of obesity in people with MDD, but accounted for only modest amount of variance. Nevertheless, as more risk loci are identified, combining a GRS approach with information on non-genetic risk factors could become a useful strategy for identifying MDD patients at higher risk of developing obesity.

6.1 Background

As noted in earlier chapters, obesity is already a serious public health problem, and obesity rates continue to rise (Wang et al., 2011). Previous studies, including Chapter 3, indicate that people with major depressive disorder (MDD) are more likely to be overweight or obese compared to psychiatrically healthy controls (Farmer et al., 2008). In addition, Chapter 3 reported that depressed people have a higher risk for various medical diseases, many of which are obesity-related. Given the high prevalence rate of both obesity and MDD, understanding the nature of their relationship is a pressing clinical problem.

Previous chapters have discussed the possibility that physiological or psychological mechanisms, possibly mediated by common genetic and environmental factors, might contribute to the overlap between obesity and MDD. In Chapter 5, genome-wide complex trait analysis (GCTA) was used to demonstrate that the phenotypic variance of both BMI and MDD is modulated by genes tagged by SNPs. Interestingly, the role of such common variants appeared more pronounced for depression than BMI. Furthermore, examination of the genetic correlation between MDD and BMI suggested a substantial overlap, although the estimate had a large standard error using bivariate GCTA as currently implemented.

In this chapter I will describe how we further aimed to investigate whether a genetic risk score (GRS), constructed using the risk variants identified in published genome-wide association studies (GWAS) on BMI, can be used to predict obesity and to

explain the variance of BMI. The advance of genome-wide association studies (GWAS) has successfully identified multiple polymorphisms associated with the risk of obesity and higher BMI (Thorleifsson et al., 2008, Willer et al., 2008, Speliotes et al., 2010). Among them, the fat mass and obesity associated (*FTO*) gene has been consistently and reliably replicated in different studies. Our team has found that several polymorphisms in the *FTO* gene, the locus conferring the highest genetic risk contribution to obesity, are associated with increased BMI in people with MDD. A disease history of depression further moderates the effect of *FTO* on BMI (Rivera et al., 2012). However, each risk variant only confers a modest effect on the risk, resulting in a limited ability for obesity prediction by applying single variants. It has been suggested that combining multiple loci into a genetic risk score (GRS) might improve prediction of obesity.

To date, several studies have examined the joint genetic effect of different numbers of identified risk variants to discriminate obesity in general population (Sandholt et al., 2010, Peterson et al., 2011, Li et al., 2010). However, to the best of our knowledge, at present no study has the GRS-based prediction of obesity in people with MDD. In this study, we aimed to investigate whether a GRS incorporating a number of well-defined common single nucleotide polymorphisms (SNPs) might have utility in prediction of obesity in patients with MDD.

6.2 Results

6.2.1 Discovery Phase

RADIANT study

Demographic characteristics

After excluding people with any missing genotype data, a total of 2,521 participants (2,086 non-obese people and 435 obese people) were included in the analysis from the RADIANT study. There were no differences in sex, age and depression status between included and excluded members of the sample (all $p > 0.05$). The mean age \pm s.d. of participants was 43.9 \pm 12.8 (non-obese people 43.2 \pm 13.1, obese people 47.3 \pm 10.7, $t = -6.08$, $p < 0.0001$). In total, 67.7% of the sample were female (72.9% of the obese sample, and 66.6% of the non-obese sample, $\chi^2 = 6.50$, $p = 0.011$). Analyses indicated that obese people were more likely to be depressed (90.3% vs 72.0%, $\chi^2 = 64.87$, $p < 0.001$).

As described in Chapter 2, the weighted and unweighted GRS for BMI was calculated from the 32 SNPs identified by Speliotes et al., (2010). The frequencies of uGRS and wGRS were approximately normally distributed (Figure 6.1). The mean uGRS (i.e. the total number of risk alleles of 32 SNPs), was 29.5 \pm 3.5 in obese people and 28.6 \pm 3.5 in non-obese people ($t = -4.47$, $p < 0.0001$). The mean wGRS was also slightly higher in obese people compared to non-obese people (4.14 \pm 0.50 vs 4.03 \pm 0.53, $t = -4.18$, $p < 0.0001$).

Principal component analysis was used to control for population stratification. The top five principal component scores were used to discriminate subpopulations of white Europeans. Principal component 1 (PC1, distinguishing southeastern European ancestry from northwestern European ancestry) and principal component 2 (PC2, distinguishing eastern European from western European ancestry) were significantly associated with BMI, and as such were included as covariates in the analysis.

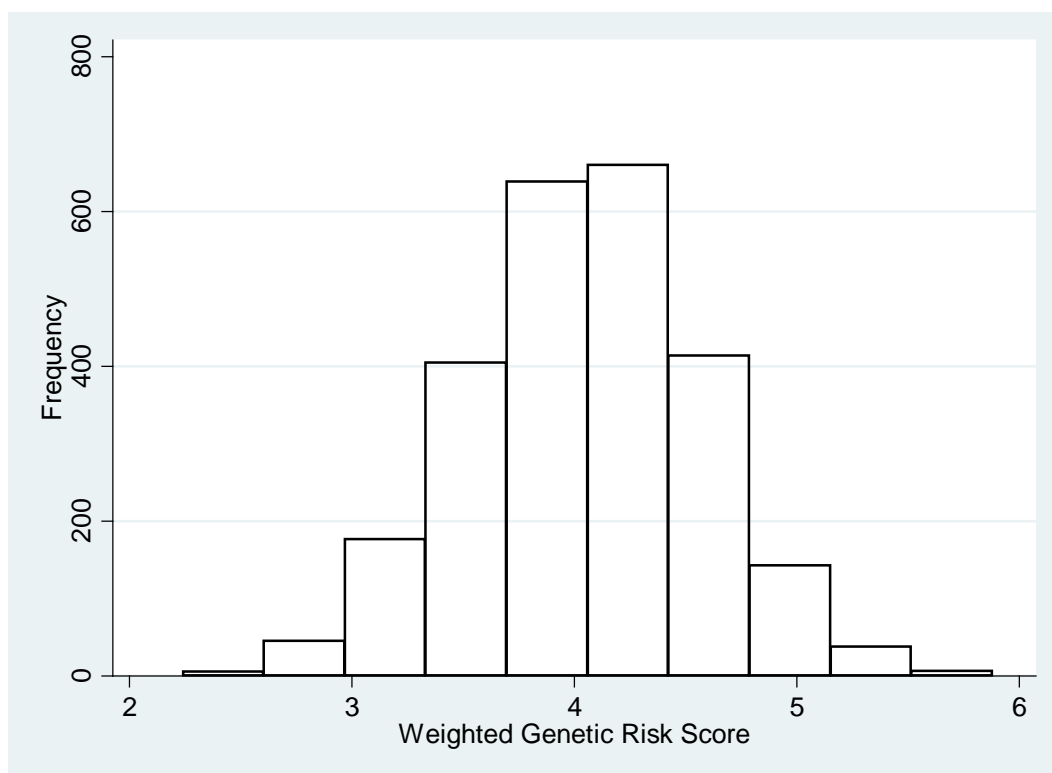


Figure 6.1 Frequencies of Weighted Genetic Risk Score

Linear regression analyses with BMI as the outcome variable

A base linear regression model including age, sex, depression status, ancestry and the interaction between ancestry and age accounted for 8.29% of the variance in log-transformed BMI. After adding the weighted GRS to the base model, there was an improvement in fit, and an additional 1.27% of phenotypic variance in BMI was explained, bringing the total to 9.56% (Table 6.1). Using either weighted or unweighted GRS made little difference to the proportion of variance in BMI explained (9.56% vs 9.58%). No interactions between traditional covariates or between GRS and traditional covariates were found (data not shown). Although the interaction between depression and GRS on BMI did not meet the conventional 5% level of significance ($\beta=0.27$, s.e.=0.02, $p=0.078$), stratifying the sample by depression status with GRS incorporated in the model explained an extra 1.63% of the variance in BMI in depressed patients, but only an extra 0.34% of the variance in BMI in healthy controls.

Table 6.1. Linear regression models with BMI as the outcome variable

Study/sample	Model	F	Adj. R ²	Additional variance explained by GRS
<i>RADIANT (Discovery phase)</i>				
Total	Model 1: adjusted by age, sex and depression	38.98	0.0829	1.27%
	Model 2: model 1+wGRS	39.16	0.0956	
Depressed cases	Model 1: adjusted by age and sex	17.85	0.0426	1.63%
	Model 2: model 1+wGRS	20.75	0.0589	
Controls	Model 1: adjusted by age and sex	11.71	0.0789	0.34%
	Model 2: model 1+wGRS	10.34	0.0823	
<i>GSK-Munich (Replication phase)</i>				
Total	Model 1: adjusted by age, sex and depression	34.02	0.1056	0.53%
	Model 2: model 1+wGRS	29.80	0.1109	
Depressed cases	Model 1: adjusted by age and sex	8.02	0.0372	1.32%
	Model 2: model 1+wGRS	7.13	0.0504	
Controls	Model 1: adjusted by age and sex	25.66	0.1306	0.23%
	Model 2: model 1+wGRS	21.98	0.1329	
<i>PsyCoLaus (Replication phase)</i>				
Total	Model 1: adjusted by age, sex and depression	40.20	0.0843	0.93%
	Model 2: model 1+wGRS	39.47	0.0936	
Depressed cases	Model 1: adjusted by age and sex	14.84	0.0605	1.09%
	Model 2: model 1+wGRS	15.15	0.0714	
Controls	Model 1: adjusted by age and sex	31.25	0.0970	0.77%
	Model 2: model 1+wGRS	29.21	0.1047	

Prediction of obesity

Logistic regression models were used to examine the relationship between GRS and obesity, in addition to age, sex, ancestry and depression status. The discriminative power of the regression model was measured by calculating the AUC. The AUC was significantly higher in the model combining all non-genetic risk factors (age, sex, ancestry and depression status) and genetic factors compared to the model only applying non-genetic risk factors (AUC increased from 0.69 to 0.71, $\chi^2=9.83$, $p=0.0017$). We further investigated whether GRS alone is able to discriminate obesity or not across depressed individuals. When only the genetic risk score and ancestry was included in the base regression model, the AUC was only 0.58 (95% CI 0.55-0.61). However, the AUC increased to 0.65 (95% CI 0.62-0.68) after adding traditional risk factors such as age and sex ($\chi^2=21.46$, $p<0.0001$). The AUC further increased to 0.71 (95% CI 0.68-0.73) when depression status was also incorporated into the model ($\chi^2=32.33$, $p<0.0001$) (Figure 6.2). Again, incorporating the unweighted GRS produced similar results to the wGRS (AUC increased from 0.58 to 0.65 to 0.70).

Similar to the analysis conducted to predict BMI, we stratified the sample based on depression status, and found that in depressed patients, the AUC increased from 0.58 (95% CI 0.55-0.61) to 0.61 (95% CI 0.58-0.64) ($\chi^2=5.65$, $p=0.0175$); whilst in healthy controls it remained at 0.67 (95% CI 0.60-0.73) ($\chi^2=0.00$, $p=0.98$). No interaction was found between depression, GRS and obesity (OR=1.08, s.e.=0.36, $p=0.81$).

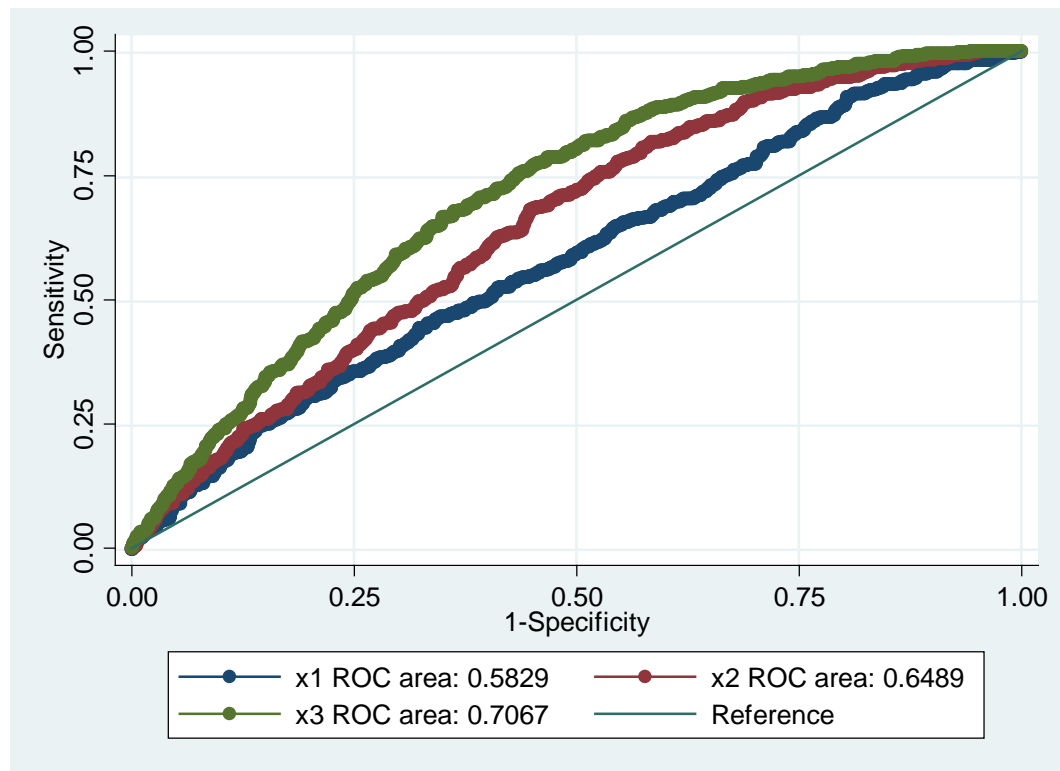


Figure 6.2 Receiver operating characteristics curves for models predicting obesity.

The AUC for full model combining depression status, age, sex and GRS is significantly greater than AUC for the model combining age, sex and GRS, which in turn is significantly greater than AUC for the base model with only GRS.

6.2.2 Replication phase

GSK-Munich study

Demographic characteristics

A total of 1,679 participants from the GSK-Munich study (244 obese people and 1,435 non-obese people) were included in this study. The mean age \pm s.d was 51.49 \pm 13.50 (53.29 \pm 11.51 for obese people and 51.19 \pm 13.80 for non-obese people, $p=0.01$). There were no gender differences between obese and non-obese people (64.75% obese people were female and 67.24% non-obese people were female, $p=0.44$). Obese people were more likely to be depressed (64.75% v. 46.27%, $p<0.001$).

Linear regression analyses with BMI as the outcome variable: data from the GSK-Munich study

The replication phase repeated analyses conducted during the discovery phase in two independent samples. First, analyses were conducted in the GSK-Munich study. Linear regression models predicting BMI suggested that the wGRS accounted for 0.63% of the variance in log-transformed BMI. When the sample was stratified by depression status, the wGRS was found to explain an extra 1.32% of phenotypic variance of BMI in depressed patients, but only accounted for 0.23% of variance in healthy controls (Table 6.1). No significant interaction was found between depression and GRS on the prediction of BMI ($\beta=0.25$, s.e.=0.01, $p=0.18$).

Prediction of obesity: data from the GSK-Munich study

Logistic regression models were used to examine the relationship between GRS and obesity in addition to age, sex, ancestry and depression status. The AUC was approximately 0.59 (95% CI 0.55-0.63) when only wGRS and ancestry were included in the base regression model. The AUC increased to 0.64 (95% CI 0.60-0.68) when traditional risk factors such as age and sex were added to the model ($\chi^2=8.21$, $p=0.004$). The AUC further increased to 0.69 (95% CI 0.66-0.73) when depression status was also incorporated into the model ($\chi^2=10.67$, $p=0.001$). Analyses on the sample stratified by depression status showed that using the wGRS to discriminate obese from non-obese individuals significantly increased the accuracy of prediction in depressed patients (AUC increased from 0.53 (95% CI 0.48-0.58) to 0.58 (95% CI 0.53-0.63), $\chi^2=4.19$, $p=0.041$), but not in healthy controls (AUC remained at 0.66 (95% CI 0.60-0.72), $\chi^2=0.34$, $p=0.56$). No significant interaction was found between depression and GRS on the prediction of obesity (OR=1.38, s.e.=0.39, $p=0.26$).

PsyCoLaus study

Demographic characteristics

2,993 subjects (409 obese people and 2,584 non-obese people) were included in PsyCoLaus study. The mean age \pm s.d is 50.19 ± 8.84 (52.94 ± 8.80 for obese people

and 49.76 ± 8.77 for non-obese people, $p < 0.0001$). There were no gender differences between obese and non-obese people (49.87% obese people are female and 53.44% non-obese people are female, $p = 0.18$). Obese people and non-obese people have equal depression rate (40.83% v. 43.69%, $P = 0.28$).

Linear regression analyses with BMI as the outcome variable: PsyCoLaus study

The second part of the replication study repeated the analysis on data from the PsyCoLaus study. Linear regression analysis to predict BMI suggested that the wGRS accounted for 0.90% of the variance in log-transformed BMI in this sample. When the sample was stratified by depression status, the wGRS explained extra 1.09% of phenotypic variance of BMI in depressed patients, but only accounted for 0.77% of variance of BMI in healthy controls (Table 6.1). No significant interaction was found between depression and GRS on BMI ($\beta = 0.09$, s.e. = 0.01, $p = 0.52$).

Prediction of obesity: PsyCoLaus study

Logistic regression models were again used to examine the relationship between GRS and obesity, in addition to age, sex, ancestry and depression status. The AUC was approximately 0.56 (95% CI 0.53-0.58) when only GRS and ancestry were included in the base regression model. The AUC increased to 0.62 (95% CI 0.59-0.65) when traditional risk factors such as age and sex ($\chi^2 = 14.61$, $p = 0.0001$) were added. The AUC remained at 0.62 (95% CI 0.59-0.65) when depression status was also incorporated into the above model ($\chi^2 = 0.11$, $p = 0.74$). Analyses on the sample

stratified by depression status demonstrated that using wGRS to discriminate obese from non-obese individuals was not statistically significant in either depressed patients (AUC increased from 0.61 (95%CI 0.56-0.66) to 0.63 (95%CI 0.58-0.67), $\chi^2=3.66$, $p=0.0558$) or in healthy controls (AUC increased from 0.61 (95%CI 0.57-0.65) to 0.62 (95%CI 0.59-0.66), $\chi^2=2.66$, $p=0.1$). No significant interaction was found between depression and GRS on the prediction of obesity (OR=0.98, s.e.=0.21, $p=0.94$).

6.3 Discussion

In this study, we developed both weighted and unweighted GRS based on 32 well established risk loci for BMI from a recent meta-analysis of GWAS (Speliotes et al., 2010). We aimed to investigate whether the GRS derived from these loci are associated with BMI and predict obesity in depressed patients and controls.

6.3.1 Prediction of BMI

Both uGRS and wGRS were associated with BMI ($p<0.0001$) and accounted for 1.27%, 0.63% and 0.90% of the phenotypic variance of BMI in the RADIANT, GSK Munich and PsyCoLaus studies, respectively. The amount of variance in BMI explained by the GRS was very similar across studies. For each unit increase in uGRS, which is equal to one additional risk allele, BMI increased by approximately 0.175 kg/m². Our overall result was thus in keeping with previous findings (Speliotes et al., 2010), which used the same method to construct a GRS for BMI, but which did

not address the relationship between BMI and depression, or the predictive utility of the GRS in depressed individuals specifically.

Our results suggest that the GRS explained more of the phenotypic variance in BMI in depressed patients compared to healthy controls, analyses of the interaction between depression status and GRS were suggestive in the discovery sample (RADIANT), but not significant in the replication samples (GSK Munich and PsyCoLaus). This could reflect the fact that significant interactions are often difficult to detect when the outcome variable has been log transformed. Interestingly the case/control difference in the effect of GRS was more prominent when depression was diagnosed in clinical settings (the RADIANT and GSK Munich studies) than in a community sample (PsyCoLaus study).

6.3.2 Prediction of obesity

We further explored the utility of a GRS approach in the prediction of obesity using a ROC analysis to compare the discriminatory ability of different predictors. Conventionally, it is accepted that the AUC in a ROC analysis should be >0.8 to be of clinical value for screening. In our discovery phase, the AUC for our predictive models fell short of this threshold, but combining genetic factors and non-genetic factors proved better than using GRS alone in the prediction of obesity (with the AUC increasing from 0.69 to 0.71). In our replication phase, findings were similar, except that depression had a small and non-significant association with obesity in the PsyCoLaus study. This could reflect the fact that PsyCoLaus was a community based study with less severe cases of MDD than the clinically ascertained RADIANT and

Munich GSK samples. Our results suggest that GRS might improve obesity prediction more in depressed patients compared to controls.

In other respects, our results were similar to those of other studies that used only genome wide significant genetic variants to construct a GRS (Sandholt et al., 2010), in finding that the optimum AUC was obtained by combining GRS and non-genetic risk factors. A significant novel feature of the present study was that combining these factors with depression status further improved the prediction of obesity. This is in keeping with the reported association between obesity and MDD in both general population or clinical settings (Farmer et al., 2008, Zhao et al., 2009, Luppino et al., 2010). In addition, the fact that GRS has a larger predictive utility on BMI and obesity in depressed patients, especially clinically severe depression suggests that genetic effects on the association between obesity and clinically significant depression may be more important at the more severe end of the distribution.

6.3.3 Limitations

Several limitations of the present study should be mentioned. First, we only selected the risk loci that reached genome-wide levels of significance (Speliotes et al., 2010) to construct the GRS. It is highly probable that there are additional, as yet unidentified loci that will emerge when even larger sample sizes are include in GWAS. Incorporating these variants into a GRS may well improve predictive utility. Second, since the common variants identified in GWAS explain only a small proportion of the variation in BMI (Chapter 6 identified the SNP heritability at 15%), future

studies should include rare variants with larger effects and copy number variants to construct the GRS. In addition, gene-gene interactions and gene environment interactions should be taken into account to maximise the predictive utility of the GRS. For example, our group (Rivera et al., 2012) has found that depression status moderates the effect of *FTO* gene on BMI, although we did not find evidence of interaction between depression and GRS in current study. Third, the 32 BMI loci we used to construct the GRS were identified in GWAS of individuals of white European origin. The allele frequencies and their effect sizes may be different in populations of non-Europeans, and our results may not be generalize to other ethnicities. Furthermore, our study is a cross sectional study, and cannot therefore take into account BMI fluctuations across the life span. A further minor limitation is that PsyCoLaus is a subset of the CoLaus study, which was one of the 46 studies from which the BMI associated risk variants was derived (Speliotes et al., 2010), and therefore cannot on its own provide an independent estimation of the risk score effect.

6.3.4 Conclusions

In summary, we found that either a weighted GRS or an unweighted GRS based on 32 well established risk loci for BMI on the basis of a meta-analysis were significantly associated with BMI in our samples. The GRS on its own explained only a small fraction of the variance in BMI, although including non-genetic risk factors together with GRS and depression status in predictive models came close to reaching the conventional threshold for clinical utility used in ROC analysis, and improved the prediction of obesity compared to GRS alone.

Our results suggest that the GRS might predict obesity prediction better in depressed patients than in healthy controls. This has potential clinical implications, as well as implications for future research directions in exploring the links between depression and obesity-associated disorders. While it is likely that future genome wide studies with very large samples will detect other than common variants, it seems probable that a combination of genetic and non-genetic information will still be needed to optimise the prediction of obesity.

Chapter Seven

Relationship between obesity and the risk of clinically significant depression: Mendelian randomisation study.

(This chapter forms the basis of a paper of the same title by Hung CF et al; that has now been published in British Journal of Psychiatry, 205(1) 24-28, 2014)

Abstract

As noted in previous chapters, obesity is associated with depression, and it has been suggested that higher body mass index (BMI) might increase the risk of depression and other common mental disorders. However, the causal relationship between BMI and depression remains unclear. Chapter 7 describes a study using Mendelian Randomisation, a form of instrumental variable analysis, to investigate whether higher BMI increases the risk of major depression. Two instrumental variable analyses were conducted to test this causal relationship in the RADIANT sample, a large case-control study of major depression. A single nucleotide polymorphism (SNP) in *FTO*, and a genetic risk score (GRS) based on 32 SNPs with well-established associations with BMI were used in the analysis.

Linear regression analysis, as expected, showed that individuals carrying more risk alleles of *FTO*, or with higher score of GRS had a higher BMI. Probit regression suggested that higher BMI was associated with an increased risk of major depression. However, our two instrumental variable analyses did not support a causal

relationship between higher BMI and major depression (*FTO* genotype: coefficient – 0.03, 95% CI –0.18 to 0.13, $P = 0.73$; GRS: coefficient –0.02, 95% CI –0.11 to 0.07, $P = 0.62$). The positive associations between higher BMI and major depression evidenced in probit regression analyses might be explained by reverse causality and/or residual confounding.

7.1 Introduction

As discussed in Chapter 1, the reasons for the well-established phenotypic association between obesity and depression are unclear. Evidence presented in Chapter 5 demonstrated that both major depressive disorder (MDD) and body mass index (BMI) have a heritable component related to common SNPs (“SNP-heritability”). The results of Chapter 5 also suggested that there is a probable genetic correlation between MDD and BMI. However, cross-sectional studies cannot rule out the possibility that there might also be a causal relationship between obesity and depression. Most previous studies have only examined the relationship between obesity and depressive symptoms, rather than depressive disorder *per se*. Although depressive symptoms could be predictors of subsequent depressive disorder, it is preferable to examine the relationship between obesity and clinically significant major depression assessed by a standardized interview. Longitudinal studies provide some pointers towards disentangling the direction of the association between the phenotypes. A recent systematic review and meta-analysis of such studies (Luppino et al., 2010) showed that obesity at the baseline increased the risk of onset of depression in the follow-up period (OR=1.55). Further, the analysis found that the association was even stronger for depressive disorder than for depressive symptoms. However, there were limitations. First, only 25% of studies included in the meta-analysis were rated as sufficiently high quality by the authors to warrant inclusion. Second, only two studies assessed a clinical diagnosis of major depression, rather than depressive symptoms only.

Amongst longitudinal studies, one report (Anderson et al., 2007) found that obese adolescent girls (but not boys) were more the likely to develop major depression twenty years later. Another study (Roberts et al., 2003) recruited people in late adulthood (mean age=63), and found that obesity increased the risk of onset of depression. However, since vascular factors plays a role in late-onset depression (Hickie et al., 2001), and given that obesity increases the risk of cardiovascular disease, the increased incidence of late-onset depression may be confounded by this shared physiological mechanism. The association between obesity and major depression might also be confounded by other unmeasured factors such as diet or exercise (Beydoun and Wang, 2010).

As described in Chapter 5, Mendelian randomization (MR) analysis has been suggested as a means to clarify causal inference in observational studies (Sheehan et al., 2008). To date, three studies have taken this approach to examine the BMI-depression association (Jokela et al., 2012, Kivimaki et al., 2011, Lawlor et al., 2011). The underlying idea is that genetic variants that are reliably associated with BMI or obesity can be used as instrumental variables (IVs) to investigate the causal effect of obesity on major depression. Recently, the advance of genome-wide association studies (GWAS) has provide the opportunity to examine the relationship between obesity and major depression, as numerous loci have been identified as risk genetic variants for BMI or obesity (Speliotes et al., 2010). Among them, the fat mass and obesity-associated (*FTO*) gene has been repeatedly and consistently associated with BMI. However, *FTO* genotype alone is not ideal for MR analysis because it only explains a very limited amount of variance in BMI. Compared to a single genetic variant, a composite genetic risk score based on multiple associated loci should be a

better instrument to examine the relationship between polygenic traits such as obesity and major depression, and should provide increased statistical power (Palmer et al., 2012). As discussed in Chapter 6, a weighted genetic risk score (wGRS) constructed from 32 single-nucleotide polymorphisms (SNPs), weighted by their effect size explained a modest but significant proportion of BMI, and inclusion of the GRS in the predictive model improved the prediction of obesity, particularly in depressed patients.

This study aimed to take an MR approach to investigate whether higher BMI increases the risk of major depression, and selecting two IVs, namely *FTO* genotype and wGRS.

7.2 Results

A total of 3,222 participants from the RADIANT study with complete BMI, age, sex and GWAS data were included in the analysis. Individuals with major depression were predominantly female, older, and had a higher BMI compared to healthy controls, but there was no difference in *FTO* genotype and wGRS between depressed patients and healthy controls (see Table 7.1). The distribution of BMI was slightly positively skewed, so BMI was natural log-transformed for further analyses. The call rate of *FTO* genotype (rs3751812) was 100%. The wGRS was obtained from 2521 participants (78.2%) with complete data for the 32 SNP genotypes. There were no differences in demographic characteristics between people with and without complete genotype data (all $P > 0.05$). Neither *FTO* genotype nor wGRS were

associated with sex ($FTO: x^2=0.17, p=0.72$; wGRS: $t=-0.93, p=0.35$) or age ($FTO: F=0.49, p=0.61$; wGRS: $cor=-.002, p=0.94$).

Both *FTO* genotype and wGRS were significantly associated with log-transformed BMI after adjusting age, sex, depression status and principal components of ancestry ($B=0.048, P=0.011$ for one risk allele in *FTO* genotype; $B=0.062, P=0.001$ for two risk alleles in *FTO* genotype; and $B=0.114, P<0.001$ for wGRS). This suggests that both *FTO* genotype and wGRS were valid indicators of risk of high BMI. A formal test for evidence against weak instruments as mentioned in the statistical analysis section (Chapter 2) confirmed these findings (F value for *FTO* genotype was 11.32, and for wGRS 33.26 in a model predicting BMI).

Results of the probit regression analysis indicated that BMI was associated with major depression (coefficient=0.05, 95% CI =0.04, 0.06, $P<0.001$). *FTO* genotype and wGRS were not directly associated with the relative risk of major depression when adjusting for covariates (1 risk allele of *FTO* genotype: coefficient=-0.06, CI=-0.17, 0.05, $P=0.31$; 2 risk allele of *FTO* genotype: coefficient=-0.01, CI=-0.15, 0.14, $P=0.93$; wGRS: coefficient=-0.03, CI=-0.14, 0.07, $P=0.54$). When using *FTO* genotype or wGRS was used as an instrumental variable for BMI, the change in BMI was not able to predict the risk of MDD (*FTO* genotype: coefficient=-0.03, 95%, CI =-0.18, 0.13, $P=0.73$; wGRS: coefficient=-0.02, 95%, CI =-0.11, 0.07, $P=0.62$) (see Table 7.2).

Table 7.1 Demographic characteristics of participants

	Depressed people (n=2430)	Healthy controls (n=792)	<i>P</i> value (X^2 or <i>t</i>)
Female	69.79 %	61.11%	$X^2=20.58$, $P<0.001$
Age	45.2 (12.2)	39.9 (13.7)	$t=-10.37$, $P<0.0001$
BMI	26.4 (5.5)	24.30 (4.5)	$t=-9.75$, $P<0.001$
<i>FTO</i> genotype			
GG	834 (34.3%)	260 (32.8%)	$X^2=1.29$, $P=0.53$
GT	1177 (48.4%)	402 (50.8%)	
TT	419 (17.2%)	130 (16.4%)	
wGRS	4.04 (0.53)	4.06 (0.51)	$t=0.56$, $P=0.57$

Table 7.2 Comparison of conventional linear and instrumental-variables regression models (with *FTO* genotype and wGRS as instrument) association of BMI and major depression

	coefficients (95% CI) for major depression		
	probit regression	IV regression (<i>FTO</i> genotype)	IV regression (wGRS)
BMI	0.05 (0.04~0.06) <i>P</i> <0.001	-0.03 (-0.18~0.13) <i>P</i> =0.73	-0.02(-0.11~0.07) <i>P</i> =0.62

7.3 Discussion

Although a positive association between obesity and major depression was found in our conventional probit regression, our independent variable analysis did not support the hypothesis that obesity increases the risk of a clinical diagnosis of major depression, using either *FTO* genotype or wGRS as the instrumental variable. Our results are not in line with the findings from other MR studies, which have suggested that obesity does have a causal influence on mental health problems. For example, the longitudinal Whitehall study (Kivimaki et al., 2011) suggested that long-term obesity is a risk factor for common mental disorder (CMD) in men. Although the Whitehall study met most of the requirements for MR analysis (Glymour et al., 2012), some important assumptions were made. First, the association between the instrumental variable, *FTO* genotype, and obesity was significant only in males but not females, in contrast to evidence from GWAS of *FTO* genotype, which have indicated associations with BMI in both genders (Speliotes et al., 2010). Second, the independent variable used in the Whitehall study might be invalid under the assumption that net unmeasured confounding is positive (Glymour et al., 2012). Third, the study did not assess CMD directly, but instead used a self-report general health questionnaire (GHQ) (Goldberg, 1972), which is a screening tool for depression and anxiety with relatively low specificity.

Another longitudinal cohort study (Jokela et al., 2012) reported that high BMI increased symptoms of depression using a genetic risk score similar to the one we used (31 SNPs). However, the association between their IV and smoking violated the

exclusion restriction assumption, as smoking might be a confounding factor between BMI and depression because there is a bidirectional relationship between smoking and depression (Chaiton et al., 2009). In addition, depression symptoms were assessed by a modified version of Beck's Depression Inventory (BDI) (Beck et al., 1996), which is essentially an instrument for measuring symptom severity rather than for assigning a diagnosis. Both MR studies found that higher BMI (obesity) increased the risk of depressive symptoms in the follow-up period. In contrast, one cross-sectional study (Lawlor et al., 2011) found that higher BMI was positively associated with psychological distress using conventional multivariable analysis, but the result was reversed while using independent variable analysis. The authors suggested that greater adiposity exerts protective effects against psychological distress via biological mechanisms, whilst the effects of stigmatization for being overweight and low self-esteem might increase the risk of psychological distress. The study was also limited in its assessment of psychological distress, which was measured using only four closed-ended questions, which have at best a modest relationship with the clinical diagnosis of major depressive disorder defined by DSM- IV (American Psychiatric Association, 2000). Our study differs from previous MR studies that mainly examined depressive symptoms or psychological distress. Instead we focused on clinically significant depression, showing that increased BMI cannot be considered as causal factor in the development of major depressive disorder severe enough to warrant treatment, and associated with a definite functional impairment.

To the best of our knowledge, our study did not violate the assumptions of an MR study (Glymour et al., 2012). First, the two independent variables used in our study,

FTO genotype and wGRS, were reliably associated with BMI and obesity, suggesting the first assumption outlined by Glymour et al., (2012) is met. Second, we did not find an association between our independent variables (genotype) and age, sex and various obesity-related physical diseases such as diabetes, hypertension and myocardial infarction, which are known to associate with major depression, suggesting that any association between *FTO* and MDD is related to obesity or high BMI. Third, for the known exposure status (i.e. BMI or obesity) and known confounders, the independent variables are independent of the outcome (major depression). However, until the underlying mechanisms linking *FTO* genotype (or GRS) and major depression are comprehensively understood, it is impossible to exclude the possibility that the second and third assumptions of MR analysis were violated.

Our probit regression analysis showed higher BMI was associated with major depressive disorder but no association was found in the independent variable analysis, suggesting either reverse causality, or the existence of important unmeasured confounders not entered into our conventional regression analysis. Unfortunately, no replicated genome-wide significant SNPs have so far been identified in GWAS of major depression (Wray et al., 2012), preventing us from carrying out an MR analysis to explore the reverse direction of causation, where depressive disorder leads to obesity. The severity of major depression in our study was at least moderate, so most participants had at some point taken antidepressant or other psychotropic agents. A recent meta-analysis (Serretti and Mandelli, 2010) suggested that some antidepressants might cause weight change during long term treatment. In addition to antidepressant induced weight change, diet and sedentary lifestyle in depressed

people might cause them to be more likely to become obese. We did not measure all the possible confounding factors such as smoking, alcohol consumption or socioeconomic status, which might also influence the association between higher BMI and major depression. Another possible explanation for our negative result is the psychological impact of obesity on the development of major depressive disorder. In Western society, obese people are more likely to be stigmatized and develop low self-esteem, resulting in the onset of depression (Puhl and Heuer, 2010). If this were the case, the association between obesity and MDD might be hypothesized to be stronger in females compared to males, and in the younger population compared to the elderly, who may be more susceptible to social stigma. However, our study did not support this explanation.

Several limitations of the present study should be considered. First, only prospective cohort studies can provide evidence regarding clear temporal relationships between risk exposures and outcomes, whereas our study was cross-sectional. Since genetic factors could influence the phenotype of BMI differently across the life-span, the choice of a relevant period of exposure may be important. However, since BMI is moderately correlated between adolescence and adulthood (Srinivasan et al., 1996, Guo et al., 2000), BMI measured at interview might be suitable as a proxy for BMI before onset of depression. This method has been applied in other Mendelian randomization studies (Lawlor et al., 2011, Mumby et al., 2011). Second, our results only pertain to the relationship between obesity and the risk of clinical depression of at least moderate severity. Therefore, we cannot rule out the possibility that obesity has a causal role in mild depression or subclinical depressive disorder. Third, our study was entirely based on white Europeans, so our results might not generalize to

other ethnicities. Fourth is the issue of power. Unfortunately, no formal methods currently exist that satisfactorily address the estimation of required sample size in MR studies (Pierce et al., 2011). However, the current sample is of a comparable or larger size than previous MR studies that have suggested a causal relationship between high BMI and depression (Kivimaki et al., 2011). Finally, a caveat pointed out by Palmer et al. (2011) is that independent variable estimators rely on the assumption of “no effect modification”, rather than the assumption of “no defiers”. However, both assumptions are impossible to test from observable data, suggesting that they are problematic to evaluate in practice.

7.4 Conclusion

Although our conventional multivariate regression analysis found that increased BMI was strongly associated with major depression, our genetic independent variable analysis did not support the hypothesis that increased BMI raises the risk of onset of major depression, using either *FTO* genotype or wGRS for high BMI as the independent variable. As described above, Mendelian randomisation studies depend on several assumptions, which are not possible to completely verify. Although our study had several limitations, the evidence presented suggests that being overweight is not an important ‘cause’ of clinically significant depression.

Chapter Eight

Further exploration of the associations between physical diseases and major depression

Abstract

Throughout this thesis, evidence for the association between MDD and various physical illnesses has been presented. This chapter takes the same approach to that used in Chapter 5 which focused on BMI and MDD to examine a) whether genetic risk loci associated with physical diseases in published studies are over-represented in depressed patients, and b) whether Mendelian randomisation analysis using risk loci as instrumental variables suggests that physical illnesses cause depression, taking asthma as an example disorder (since the results of Chapter 2 suggested that the phenotypic overlap between asthma and depression may have a molecular genetic underpinning). The results indicate that the SNP rs1342326, which is near the *IL-33* gene, and is reportedly significantly and consistently associated with asthma in GWAS, is over represented in patients with MDD. However, the Mendelian randomisation analysis using two independent variables, namely rs1342326 and a weighted genetic risk score (wGRS) for asthma, did not support a direction of causality from asthma to depression. Therefore, the results suggest that there is a small but significant genetic overlap between the liabilities to depression and to

asthma, which may contribute to the excess of asthma in depressed subjects compared to controls.

8.1 Introduction

As discussed in Chapter 1, recent studies (Farmer et al., 2008) including my own (Chapter 3) have shown that MDD and physical diseases are frequently co-morbid. Prevalence rates of major depressive disorder are significantly higher in people with a chronic medical condition than those without (Fiest et al., 2011). As described in Chapter 1, previous work from our group (Farmer et al., 2008) found the highest three most prevalent physical diseases in people with recurrent depression are asthma, hypertension, and hypercholesterolemia, after controlling for age, sex and body mass index (BMI). Analyses presented in Chapter 3 demonstrated that, controlling for the same variables, hypertension, hypercholesterolemia and myocardial infarction were also over represented in depressed people, as were asthma and allergic rhinitis. The nature and directionality of the relationship between these physical diseases and MDD, and whether the specific attributes of the diseases contribute to the causation of MDD, remains unclear.

As noted previously, GWAS have been designed to detect the common genetic variants that are associated with complex traits or diseases (Hirschhorn and Daly, 2005), and although thousands of risk loci have been identified, the number of loci identified per complex trait varies greatly (Visscher et al., 2012), with no replicated genome-wide hits yet identified for MDD (Major Depressive Disorder Working Group of the Psychiatric GWAS Consortium, 2012). The reasons for these negative results are discussed in detail Chapter 1.

One consequence of the absence of genome wide significant ‘hits’ for depression is that, at present, it is not possible to explore whether depression has a molecular genetic overlap with disease X simply by examining whether depression and disease X have genome wide significant risk loci in common. There are, however, strategies that can exploit genome wide data on depression to examine the molecular genetic overlap with other diseases that do not require the stringent significance levels employed in a genome wide search.

The first is simply to take polymorphisms that have been robustly associated with each of the diseases showing a phenotypic overlap with depression and test them for depression case/control differences. Such an analysis requires correction for a much smaller number of comparisons, and is therefore much more powerful than analyses requiring genome-wide levels of correction. The second and slightly more complex approach is to construct a genetic risk score (GRS) for each disease associated with depression, using the method described in Chapter 5, and see whether that GRS shows any association with depression. A third approach, which gives some purchase on the direction of causation, is to use individual disease associated loci and/or a GRS in an instrumental variable in a Mendelian randomisation analysis. This method was used to examine the causal link between high BMI/obesity and depression in Chapter 6. A fourth approach is to use a bivariate genome wide complex trait analysis (GCTA), to explore the genetic correlation between depression and “Disease X”, the method used to explore the relationship between BMI and depression in Chapter 4. A fifth approach would be to use polygenic score analysis and test whether a score derived from a GWAS of “disease X” predicts depression.

Unfortunately, the fourth and fifth approaches mentioned here require access to genome wide data on “disease X”, which we have not been able to obtain for any of the diseases shown to be phenotypically associated with depression in Chapter 3. We therefore have to rely on published data on risk alleles associated with these disorders, and use the second and third approaches described above to investigate the relationship and direction of causality.

In Chapter 3, eight diseases are associated with MDD were identified, namely asthma, allergic rhinitis, hypertension, hyperlipidemia, myocardial infarction, gastric ulcer, osteoarthritis and thyroid disease. As described below (Methods section), only asthma was suitable for further analysis in our dataset. Although gastric ulcer was among the first disease to be associated with genetic polymorphisms (Edwards, 1965), no GWAS on gastric ulcer have been conducted. In addition, data from the RADIANT study used in these analyses did not separate hypercholesterolemia into HDL-cholesterol or LDL-cholesterol, and did not separate thyroid disease into hyper- or hypothyroidism, which prevents us from constructing a GRS for further investigation of these conditions. There are only a few GWAS identifying risk SNPs reaching genome-wide significance for allergic rhinitis (Ramasamy et al., 2011), hypertension (Ehret et al., 2011, Padmanabhan et al., 2010, Levy et al., 2009), osteoarthritis (Zeggini et al., 2012, Betancourt et al., 2012) and myocardial infarction (Aoki et al., 2010, Helgadottir et al., 2007). Therefore, of the diseases overrepresented in depressed cases, asthma was the only candidate disease suitable for this approach.

The aim of this study was to examine whether individual polymorphisms associated with physical diseases are overrepresented in our sample of depressed individuals. A GRS for asthma was then calculated. Finally, an analysis using Mendelian Randomization (MR) was conducted, given that the analysis of GWAS data suggested that the phenotypic overlap of asthma and MDD may have a molecular genetic underpinning.

8.2 Results

A total of 22 studies including 2 studies on myocardial infarction (Aoki et al., 2010, Helgadottir et al., 2007), 3 studies on hypertension (Ehret et al., 2011, Padmanabhan et al., 2010, Levy et al., 2009), 5 studies on asthma (Heath and Madden, 2012, Wan et al., 2012, Ferreira et al., 2011, Moffatt et al., 2010, Ober and Nicolae, 2011), 1 study on allergic rhinitis (Ramasamy et al., 2011), 4 studies on obesity (Bradfield et al., 2012, Jiao et al., 2011, Wang et al., 2011, Meyre et al., 2009) and 7 studies on BMI (Frayling et al., 2007, Loos et al., 2008, Willer et al., 2008, Thorleifsson et al., 2008, Speliotes et al., 2010, Wen et al., 2012, Yang et al., 2012) were included in our analysis. All these studies fulfilled the requirement of having at least one hit reaching genome-wide significance for a physical illness. One hundred and seven hits were found in these studies, and seven hits (rs7775228, rs2155219 and rs17513503 on allergic rhinitis; rs6545814, rs9356744, rs261967 and rs1297579 on BMI) that were not replicated in the same or in other independent studies were excluded. After excluding the SNPs in LD with each other ($r^2 > 0.5$), 60 SNPs survived. When the prevalence of SNPs in cases and controls with MDD was examined, one hit (rs1342326 located at 9p24.1, intergenic region between *RANBP6* and *IL-33* gene)

reached the threshold for statistical significance ($p < 8.3 \times 10^{-4}$) after correction for multiple testing (see Table 8.1).

To test whether asthma *per se* might be a risk factor for MDD, formal MR analysis was conducted using our RADIANT data. A total of 2692 participants (1887 cases and 805 controls) were included in the analysis. The depressed patients were older than healthy controls (mean age (SD): 46.1(12.2) v. 39.9(13.7), $t=11.61$, $P<0.0001$) and there were more females among cases (72.28% v. 61.37%, $\chi^2=31.45$, $P<0.001$). However, no difference in rs1342326 genotype and wGRS was observed between cases and controls (Table 8.2). The frequency of wGRS was approximately normally distributed (Figure 8.1).

The relationship between asthma and wGRS/ rs1342326 genotype was examined using logistic regression controlling for age, sex, ancestry and depression status.

Both wGRS and rs1342326 genotype increases the risk of asthma (wGRS: odds ratio=1.10 (95% CI=1.05-1.15, $z=4.41$, $P<0.001$; rs1342326: odds ratio=1.31 (95% CI=1.05-1.64), $z=2.37$, $P=0.018$). Logistic regression indicated that asthma is associated with MDD (odds ratio=1.79 (95% CI=1.35-2.37), $z=4.06$, $P<0.001$).

However, we found that both rs1342326 genotype and wGRS are weak instrumental variables (IVs) (F value for rs1342326= 8.82 and F value for wGRS=6.64). Using these two IVs for asthma, it appears that the presence of asthma cannot predict the risk of MDD (Table 8.3).

8.1 Physical diseases/ complex traits with genetic variants reaching genome-wide significance

Disease/ Traits	Region	Reported Genes	Strongest SNP	Risk allele	Proxy SNP in PGC MDD	p-value (MDD case- control difference)	Corrected p-value	Study
Asthma	1q21.3	IL6R	rs4129267	T		0.922	n.s	Ferreira, 2011
Asthma	2q12.1	IL18R1	rs3771166	G		0.05123	n.s	Moffatt, 2010
Asthma	2q12.1	IL1RL1	rs3771180	?		0.1696	n.s	Heath, 2012, Torgerson, 2011
Asthma	4q31.21	LOC729675	rs7686660	T		0.7777	n.s	Hirota, 2011
Asthma	5q22.1	TSLP	rs1837253	?		0.4892	n.s	Torgerson, 2011
Asthma	6p21.32	HLA-DQ	rs9273349	C		0.4434	n.s	Moffatt, 2010
Asthma	9p24.1	IL33	rs1342326	C		0.0003872	0.023	Moffatt, 2010; Torgerson, 2011
Asthma	11q13.5	LRRC32	rs7130588	G		0.2899	n.s	Ferreira, 2011
Asthma	12q13.2	IKZF4	rs1701704	G		0.2694	n.s	Hirota, 2011
Asthma	15q22.33	SMAD3	rs744910	G		0.05768	n.s	Moffatt, 2010
Asthma	17q12	GSDMB	rs11078927	?		0.259	n.s	Torgerson, 2011
Asthma	17q21.1	GSDMA	rs3894194	A		0.05554	n.s	Moffatt, 2010
Asthma	22q12.3	IL2RB	rs2284033	G		0.8971	n.s	Moffatt, 2010
BMI	1p21.3	PTBP2	rs1555543	C		0.1437	n.s.	Speliotes, 2010
BMI	1p31.1	NEGR1	rs2568958	A		0.0763	n.s	Thorleifsson, 2008PTBP2
BMI	1p31.1	TNNI3K	rs1514175	A		0.1088	n.s	Graff, 2013
BMI	1q25.2	SEC16B	rs51663	A		0.2173	n.s	Okada, 2012

BMI	1q25.2	SEC16B	rs543874	G		0.3088	n.s	Speliotes 2010, ;Graff, 2013
BMI	2p23.3	ADCY3, DNAJC27	rs6545814	G		0.2211	n.s	Wen, 2012
BMI	2p25.3	TMEM18	rs2867125	C		0.307	n.s	Speliotes, 2010
BMI	3p12.1	CADM2	rs13078807	G		0.822	n.s	Speliotes, 2010
BMI	3q27.2	SFRS10, ETV5, DGKG	rs7647305	C		0.0012	n.s	Thorleifsson , 2008
BMI	4p12	GNPDA2	rs10938397	G	rs12641981 (r ² =0.967)	0.4433	n.s	Speliotes, 2010
BMI	5q13.3	FLJ35779, HMGCR	rs2112347	C	rs3797580 (r ² =1)	0.1794	n.s	Speliotes, 2010
BMI	5q15	PCSK1	rs261967	C	rs261966 (r ² =0.966)	0.8192	n.s	Wen, 2012
BMI	5q23.2	ZNF608	rs4836133	A	rs6864049 (r ² =1)	0.1171	n.s	Speliotes, 2010
BMI	6p12.3	TFAP2B	rs987237	G		0.6304	n.s	Speliotes, 2010
BMI	6p22.3	CDKAL1	rs2206734	T		0.8636	n.s	Wen , 2014
BMI	6p22.3	CDKAL1	rs9356744	T	rs1569699 (r ² =0.96)	0.7629	n.s	Wen, 2012
BMI	9p21.1	LRRN6C	rs10968576	G		0.04162	n.s	Speliotes, 2010
BMI	9q21.12	KLF9	rs11142387	C		0.9809	n.s	Okada, 2012
BMI	11p11.2	MTCH2	rs10838738	G		0.2232	n.s	Willer, 2009
BMI	11p14.1	BDNF	rs10767664	A	rs7103411 (r ² =1)	0.1258	n.s	Speliotes, 2010
BMI	11p14.1	BDNF	rs925946	T		0.1559	n.s	Thorleifsson , 2008
BMI	11p15.4	RPL27A, TUB	rs4929949	C	rs9300092 (r ² =0.966)	0.07818	n.s	Speliotes, 2010
BMI	12q13.12	FAIM2	rs7138803	A		0.875	n.s	Speliotes, 2010
BMI	15q23	MAP2K5, LBXCOR1	rs2241423	G		0.06558	n.s	Speliotes, 2010
BMI	16p11.2	SH2B1	rs7498665	G		0.4698	n.s	Thorleifsson , 2008

BMI	16q12.2	FTO	rs12149832	A	rs9930506 (r ² =0.935)	0.06984	n.s	Okada, 2012
BMI	16q12.2	FTO	rs6499640	A		0.6721	n.s	Thorleifsson, 2008
BMI	16p12.3	GPRC5B, IQCK	rs12444979	C	rs6497416 (r ² =1)	0.9492	n.s	Speliotes, 2010
BMI	18q21.32	MC4R	rs17782313	C		0.03726	n.s	Loos, 2008
BMI	18q21.32	MC4R	rs571312	A		0.03037	n.s	Speliotes, 2010
BMI	19q13.11	KCTD15	rs29941	G		0.122	n.s	Speliotes, 2010
BMI	19q13.32	GIPR	rs11671664	G		0.119	n.s	Okada, 2012
BMI	19q13.32	TMEM160, ZC3H4	rs3810291	A		0.8589	n.s	Speliotes, 2010
obesity	10q22.3	KCNMA1	rs2116830	G		0.8177	n.s	Jiao, 2011
obesity	13q14.3	OLFM4	rs9568856	?	rs4883723 (r ² =0.925)	0.1701	n.s	Bradfield, 2012
obesity	16q12.2	FTO	rs17817449	?		0.03192	n.s	Wang, 2011
obesity	16q23.2	MAF	rs1424233	A		0.8452	n.s	Meyre, 2009
obesity	17q21.32	HOXB5	rs9299	?		0.5596	n.s	Bradfield, 2012
Hypertension	6p12.3	UMOD	rs1333226	G		0.1975	n.s	Padmanabhan, 2010
Hypertension	12q21.33	ATP2B1	rs2681472	A		0.7365	n.s	Levy, 2009
Myocardial infarction	5p13.3	NPR3, C5orf23	rs1173771	G		0.0658	n.s	Ehret, 2011
Myocardial infarction	5p15.33	Intergenic	rs11748327	?	rs2047074 (r ² =0.881)	0.7528	n.s	Aoki, 2010
Myocardial infarction	6p21.33	BAT2, BAT5	rs805303	G		0.06956	n.s	Ehret, 2011
Myocardial infarction	9p21.3	CDKN2A, CDKN2B	rs10757278	G	rs4977574 (r ² =1)	0.6727	n.s	Wahstrand, 2009
Myocardial infarction	10q23.33	PLCE1	rs932764	G	rs10786152 (r ² =0.845)	0.9723	n.s	Ehret, 2011
Myocardial infarction	11q22.1	FLJ32810, TMEM133	rs633185	G	rs604723	0.1016	n.s	Ehret, 2011

				($r^2=1$)			
Myocardial infarction	20q13. 32	GNAS, EDN3	rs60154 50	G	0.2671	n.s	Ehret, 2011

Table 8.2 Demographic Characteristics of Participants

	Depressed people (n=1887)	Healthy controls (n=805)	<i>P</i> value (X^2 or <i>t</i>)
Female	72.28 %	61.37%	$X^2=31.45$, $P<0.001$
Age	46.1 (12.2)	39.9 (13.7)	$t=-11.61$, $P<0.0001$
Asthma	19.28%	11.43%	$X^2=24.79$, $P<0.001$
rs1342326 genotype			
CC	51 (2.7%)	20 (2.5%)	$X^2=0.998$, $P=0.61$
CA	505 (26.8%)	202 (25.1%)	
AA	1330 (70.5%)	583 (72.4%)	
wGRS	14.51 (2.98)	14.53 (3.06)	$t=0.19$, $P=0.84$

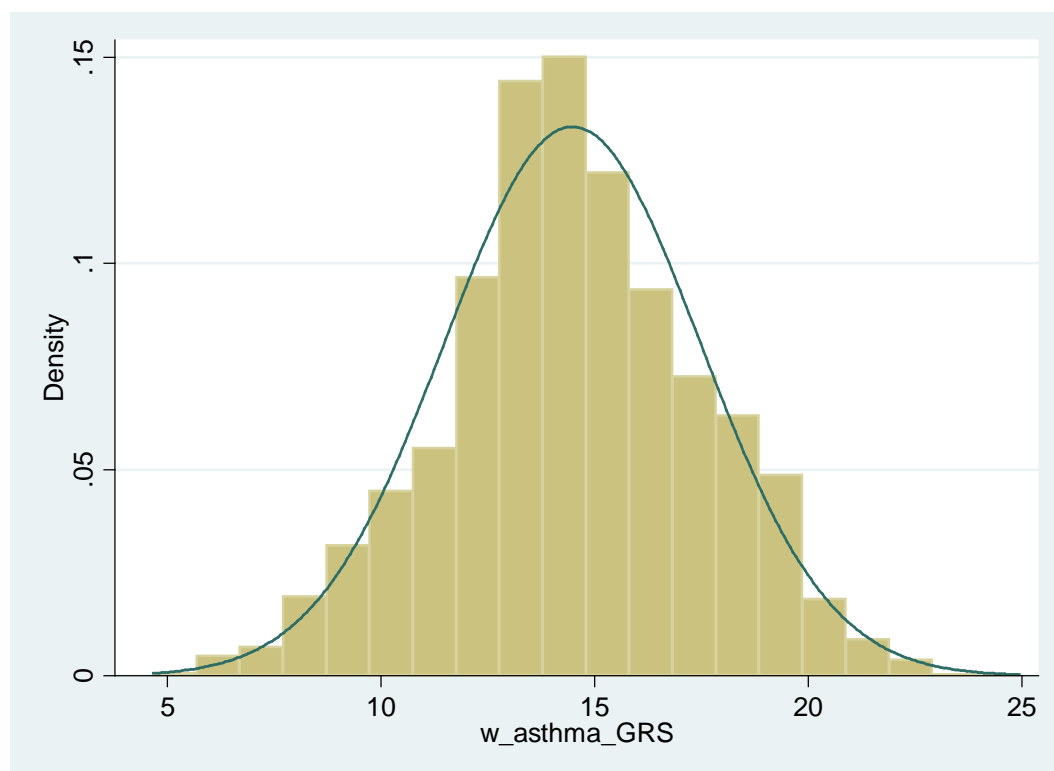


Figure 8.1 Frequency of weighted Genetic Risk Score (wGRS) for asthma

Table 8.3 Comparison of Conventional logistic and Instrumental-Variables Regression Models (with rs13422326 Genotype and wGRS as Instrument) examining the association between asthma and Major Depression

Odds ratio/ Coefficient(95% CI) for major depression			
	logistic regression	IV regression (rs1342326)	IV regression (wGRS)
Asthma	1.79 (1.35, 2.37)	0.87(-1.43, 3.18)	0.49 (-2.30, 3.29)
	<i>P</i> <0.001	<i>P</i> =0.36	<i>P</i> =0.73

The F value for rs1342326 was 8.82, and the F value for wGRS was 6.64. As such, both were weak instrumental variables ($F < 10$).

8.3 Discussion

This study, which used published GWAS data on physical illnesses and MDD, as well as data from our own group, attempted to clarify their relationship between physical illnesses and MDD at the molecular level. Only asthma had a risk polymorphism that was also associated with depression after correcting for multiple testing. The asthma risk variant which had a statistically significant difference between MDD cases and controls was rs1342326, which is located between the *RANBP6* and *IL-33* genes. The C allele of the rs1342326 polymorphism is associated with an increased risk of asthma, and was also over-represented in patients with MDD. Because the transmission of genetic variants is randomly distributed during the period of gamete formation, the association between rs1342326 and MDD should be largely independent of confounding factors. As such, analysis used these asthma-risk genotypes to examine whether asthma might be a risk factor for developing major depression. However, this Mendelian Randomisation analysis conducted in our RADIANT data failed to provide evidence that asthma raises the risk of MDD. The results therefore suggest that the asthma associated allele of rs1342326 is a probable risk factor for depression, but that asthma itself is not a risk factor for depression. That said, both rs1342326 and wGRS are weak instrumental variables, which may have resulted in a bias in the two-stage least square estimator, even though the analysis was conducted in a large sample (Staiger and Stock, 1994).

Both asthma and MDD are two of the most common chronic medical conditions in the world. The lifetime prevalence rate of MDD has been estimated as approximately

16% in US (Kessler Rc and et al., 2003) whereas the current prevalence rate of asthma is approximately 8% and increased steadily in US (Akinbami et al., 2011). Studies have shown that individuals with asthma suffer more from depressive symptoms or MDD in either clinical settings or general population (Nejtek et al., 2001, Goodwin et al., 2004, Ng et al., 2007). Other studies (Farmer et al., 2008, Loerbroks et al., 2012), including my own (Chapter 2) indicate that depressed patients are also more likely to have asthma. Although there is solid evidence suggesting that asthma is associated with MDD, the causal relationship between these two diseases remains unclear. To clarify the causal relationship between asthma and MDD, it would be useful to investigate their temporal relationship. Since MDD often develops in adulthood, less than a quarter of patients who have co-morbid asthma and MDD develop MDD prior to asthma onset (Solis et al., 2006). One of possible explanation of the generally later onset of MDD than asthma is that having asthma is itself a risk factor for MDD. Unfortunately, the majority of the previous association studies examining the relationship between these two diseases are cross-sectional, and cannot provide enough information to detangle the causal relationship. Recently, a longitudinal study using a UK research database to follow up more than ten thousand people for ten years found that the incidence of depression at baseline in people with asthma was 22.4/1,000 person years, compared with 13.8/1,000 person years in people without asthma (Walters et al., 2011). This study provides further evidence that asthma might be a risk factor for depression from the epidemiological viewpoint.

Nevertheless, the underlying mechanisms driving the association between asthma and depression remain unclear. Several candidate pathways have been suggested

comprising both genetic factors and environmental factors affecting autonomic nervous system and/or immune system dysregulation (Van Lieshout et al., 2009). A twin study estimated that 64% of the association between atopy and depression was due to shared familial vulnerability, consisting mainly of additive genetic factors (Wamboldt et al., 2000). The asthma associated marker we identified that was associated with depression is close to the gene encoding interleukin-33 (*IL-33*), a novel member of IL-1 cytokine family (Liew et al., 2010). *IL-33* can be both a secreted cytokine and a nuclear binding factor, although its precise function as a nuclear factor remains unclear. It is expressed in various human cells, but its function is thought to vary across different tissues. *IL-33* signals via a heterodimer receptor complex consisting of ST2 and IL-1R accessory protein. Higher concentrations of *IL-33* protein have been found in patients with chronic asthma, and animal studies has shown that *IL-33* exacerbate experimental asthma in mice (Louten et al., 2011). A recent GWAS study (Moffatt et al., 2010) has also reported an association between *IL-33* and asthma.

Notably, *IL-33* and its receptor ST-2 are also widely expressed in human adipose tissue. The *IL-33*/ST-2 pathway plays a critical role in glucose homeostasis and protects against adipose tissue inflammation and atherosclerosis (Miller et al., 2008). *IL-33* and *ST-2* are also expressed in various tissues within the central nervous system, and *IL-33* may have a pathogenic role in inflammatory diseases of the CNS. A polymorphism of *IL-33* is also associated with an increased risk of Alzheimer's disease (Morimoto et al., 2011). In addition, *IL-33* can induce inflammatory pain in peripheral nervous system.

Although no previous reports have investigated the relationship between IL-33 and major depression, another IL-1 family member, IL-1 β has been reported to be a major regulator of the central inflammatory cascade. IL-1 β can regulate several genes expressed in the brain, including CRH receptor 1 (CRHR1) gene, which has been associated with treatment response to antidepressants (Licinio et al., 2004). Depending the type of tissue, IL-33 can either initiate or maintain the inflammatory process, or resolve the inflammatory state.

Although an association between inflammation and depression or depression-like symptoms has been established, the exact biological mechanisms remain unknown. Inflammation appears to account for a small proportion of the variance in depression in patients with physical diseases, rather than being a major factor in the majority of cases (Dantzer et al., 2008). Mechanisms that have been suggested to explain inflammation-related depression, irrespective of whether overt physical disease is also present, include induction of extra hypothalamic corticotrophin, development of glucocorticoid resistance, activation of the kynurenine pathway and increased expression of the serotonin transporter (Miller, 2009). Among these, activation of the kynurenine (KYN) pathway is perhaps the most plausible mechanism. The kynurenine pathway is a major route of tryptophan catabolism, and the two main oxygenases responsible for degradation of tryptophan are tryptophan dioxygenase (TDO) and indoleamine 2,3-dioxygenase (IDO). IDO is the rate-limiting enzyme of kynurenine pathway in all extrahepatic tissues. In the brain, microglial cells can

express IDO by the stimulation of interferon-gamma (Kwidzinski and Bechmann, 2007) and tumor necrosis factor (TNF- α).

KYN may also have a role in depression in patients with cancer (Stone and Perkins, 1981) and in other disorders where there is peripheral inflammation. Some evidence for this hypothesis comes from studies of interferon-gamma induced depression in patients with hepatitis C. Depression induced by interferon-gamma has been found to be correlate with peripheral tryptophan metabolites and KYN metabolites in plasma and in cerebrospinal fluid (Raison et al., 2010).

Before concluding, several limitations of this study need to be considered. First, suitable single genetic variants to use as instrumental variables for Mendelian randomization studies are not easy to find. The effect size of these genetic variants tends to be extremely small, and may not be detectable despite large sample sizes. In addition, major depression is a probably a heterogeneous disease. Although we have shown that physical diseases are associated with major depression, we have not determined whether this applies to certain types of major depression. Furthermore, our results suggesting that asthma is a risk factor for depression does not exclude the possibility of a reverse causal relationship, or a shared physiological pathway. Third, the formal test of Mendelian randomisation analysis using two IVs for asthma did not support a direction of cause effect from asthma to depression. Therefore, the results only suggest that there is a small but significant genetic overlap between the liabilities to depression and to asthma that may contribute to the excess of asthma in depressed subjects compared to controls.

Chapter Nine

Discussion and Conclusions

The overall aim of my PhD study was to investigate the relationship between major depressive disorder (MDD) and a range of common physical diseases, plus obesity. Although both MDD and common physical diseases cause significant amount of disease burden, it is only comparatively recently that studies have begun to place much emphasis on their co-morbidity. In addition to investigating the prevalence rates and familial aggregation of physical diseases in depressed people, this thesis aimed to disentangle the relationship between MDD and physical diseases using various genetic approaches. Below, I will briefly consider my main research questions and principal findings.

9.1 Review of Research Questions

Question 1: Are physical diseases more prevalent in patients with major depressive disorder and their siblings and what is the pattern of physical disease resemblance for physical diseases in sib-pairs with depression?

In my first study (Chapter 3), I attempted to replicate previous findings from our group indicating that physical diseases are more prevalent in individuals with MDD (Farmer et al., 2008). Analyses were conducted on an independent sample, the

Depression Network study (DeNT), to examine the hypothesis that there are higher prevalence rates of various physical diseases in depressed people. One strength of the DeNT study sample is that it consists of sibling pairs with depression. This allowed me to further examine familial aggregation of physical diseases and their inter-correlation in depressed families.

The results indicated that eight out of 16 common chronic physical diseases were more prevalent in individuals with MDD after controlling age, sex, body mass index (BMI), depression status and family membership. The following disorders were significantly more frequent in depressed patients: hypertension, hypercholesterolemia, myocardial infarction, asthma, allergic rhinitis/ hay fever, gastric ulcer, osteoarthritis and thyroid disease. The results are largely in line with the previous report from our group (Farmer et al 2008), except that two additional depression-associated conditions, namely hypercholesterolemia and myocardial infarction, were identified in my analysis. In addition, not only affected siblings, but also unaffected siblings of depressed patients had similarly high prevalence rates for various physical diseases. Notably, hypertension, hypercholesterolemia, asthma, osteoarthritis and gastric ulcer were familial in depressed patients. Factor analysis on disease patterns across the sample suggested that there were six groups of disorders, with the most significant factor relating to a 'metabolic syndrome' group, which consisted of type 2 diabetes, hypertension, hypercholesterolemia and myocardial infarction.

Our data indicated that, in line with other studies, obesity was more common in depressed patients. Increased BMI has been shown to associate with various physical diseases. Our analyses suggested that the strong association between MDD and various physical diseases, especially metabolic related diseases, is partly due to increased body mass index. However, eight physical diseases were associated with MDD even taking account of BMI and other confounding factors. Familial aggregation of various physical diseases in depressed people might reflect the potential common genetic contribution to these diseases, although our sib-pair study design cannot disentangle the extent of genetic influences on physical diseases in depressed patients from environmental contributions.

In summary, the study described in Chapter 3 suggested that there is a higher prevalence rate of various chronic common physical diseases in depressed families. Several diseases, especially metabolic related physical diseases, clustered in MDD families. Questions about the role of causal pathways and common genetic factors underpinning the relationship between obesity/ physical diseases and depression was explored in later chapters.

Question 2: Do personality traits influence the co-morbidity between physical diseases and major depression, and is there any familial correlation between asthma and neuroticism?

Previous studies have shown that there is a relationship between personality traits and MDD. Certain personality traits, for example neuroticism, are associated with the development of MDD (Farmer et al., 2002) and the prognosis of MDD (Ranjith et al., 2005). In addition, some studies have suggested that personality traits are associated with various physical diseases (Charles et al., 2008). Chapter 4 aimed to explore whether the association between MDD and various physical diseases was driven, at least in part, by personality traits.

As in Chapter 3, analyses were conducted in the DeNT sample. Results indicated that neuroticism was modestly but significantly positively associated with the number of physical diseases, while extraversion and psychoticism were negatively associated with the number of physical diseases. However, the effect sizes were small and only neuroticism was still significantly associated with the number of physical diseases after taking into account depressive status, sex, age and BMI. A similar pattern was found when the relationship between personality traits and the presence of any physical disease was examined: neuroticism was modestly positively associated with the presence of any physical disease.

To determine whether neuroticism was associated with any of the specific physical diseases under study, the analysis was repeated for each disease separately. Only asthma was significantly associated with neuroticism, and again the coefficient was small. Subsequently, bivariate structural equation modelling was used to explore whether there is familial overlap between asthma and neuroticism. The model I tested made a number of assumptions, including using published parameter estimates for the familiarity of asthma. However, model comparison indicated that the parameter estimating the familial correlation between asthma and neuroticism could

be dropped from the model without worsening the fit, suggesting that the association between higher neuroticism score and asthma is unlikely to reflect familial factors common to these two traits.

In summary, this study was in agreement with previous reports suggesting that neuroticism is associated with an increased number of reported physical diseases/complaints, although the magnitude of the effect is small. The bivariate modeling analysis did not support the hypothesis that there is a familial correlation between asthma and neuroticism. Notably, because the study was conducted in a sample of depressed patients, restricted variance on trait neuroticism may have contributed to this negative finding.

Question 3: What is the ‘SNP heritability’ of major depression and body mass index estimated from a large case control study, and is there a genetic correlation between these two traits that is tagged by common variants?

The results of Chapter 4 suggested that the personality trait of neuroticism does not play a major role in the relationship between physical diseases and MDD. Obesity on the other hand contributes significantly to numerous physical diseases, especially metabolic related diseases, in individuals with MDD. This motivated an investigation using GWAS data to estimate the heritability of both MDD and body mass index, and examine the genetic correlation between these two trait/ diseases. This analysis used a new analysis method called genome-wide complex trait analysis (GCTA)

(Yang et al., 2011) developed to investigate the phenotype variance explained by common genetic variants captured by microarray platform, often now referred to as ‘SNP heritability’. Furthermore, a bivariate extension of this method makes it possible to examine the genetic correlation between the two diseases/ traits using GWAS data.

Analyses were conducted using data from the RADIANT studies, which include DeNT (Depression Network Study), DeCC (Depression case control study) and GENDEP (Genome-based Therapeutic Drugs for Depression), in order to enlarge the sample size to get better estimates using GCTA. An additional independent sample, the Munich-GSK depression case control study, was added to these data to further increase power. Results based on analyses conducted in the RADIANT sample suggested that the additive genetic variance of BMI and MDD related to common SNPs were 0.17 (S.E.=0.12, $P=0.10$) and 0.36 (S.E.=0.12, $P=0.001$), respectively. The estimated genetic correlation between BMI and MDD was 0.61 (SE=0.26, $P=0.02$). On combining the Munich and RADIANT samples, variance in BMI attributable to common SNPs was 0.15 (SE=0.09, $P=0.04$), and variance in MDD was 0.33 (SE=0.08, $P=5*10^{-6}$). However, the estimated genetic correlation between BMI and MDD was no longer significantly different from zero, due to the large standard error ($rG=0.40$, SE=0.23, $P=0.08$). These results indicate that a substantial proportion of phenotypic variance of both MDD and BMI can be explained by common genetic variants. With respect to MDD, it appears that there is actually little ‘missing heritability’ compared with estimates of heritability derived from population based twin studies (Sullivan et al., 2000). This is of interest in particular for MDD, where GWAS approaches have so far been singularly unsuccessful in identifying any consistent genome-wide ‘hits’ (Wray et al., 2012). Analyses based on

the RADIANT study data further indicated a substantial and significant genetic correlation between BMI and MDD. Unfortunately, the estimate was imprecise with large confidence interval, and the addition of a smaller dataset provided by colleagues in Munich rendered the genetic correlation non-significant by the conventional criterion of $p < 0.05$. However, the results remain somewhat suggestive of a genetic correlation between BMI and MDD and are therefore certainly require investigation in larger samples.

Question 4: To what extent can genetic risk score for BMI constructed using identified risk SNPs from a large meta-analysis of GWAS explain the variation in BMI in depressed patients and improve the prediction of obesity in individuals with major depressive disorder?

One paradox of the findings in Chapter 4 suggesting that MDD actually has a higher SNP heritability than BMI is that published GWAS of BMI and obesity have so far actually been more successful than GWAS of MDD in identifying risk loci that are genome wide significant and replicable. The analyses reported in Chapter 5 capitalised on these identified risk loci for BMI identified from a large meta-analysis of GWAS to construct a genetic risk score (GRS). This was used to estimate the extent to which variation in BMI can be explained by the GRS in a sample of depressed patients. Analyses also examined whether the GRS was a useful predictor of obesity in individuals with MDD, both on its own and in combination with other measured risk factors.

A large GWAS of BMI identified 32 SNPs at genome-wide significance (Speliotes et al., 2010). Two GRS were constructed on the basis of the number of risk alleles for these loci in patients within our sample. Both un-weighted GRS (uGRS, the count of risk alleles), and weighted GRS (wGRS, the sum of the the products of each risk allele multiplied by its effect size), were calculated. The frequencies of uGRS and wGRS were approximately normally distributed. Both the mean uGRS and wGRS were higher in obese people compared to non-obese people. Demographic predictors of high BMI consisting of age, sex and depression status together explained 8.29% variance of BMI, and adding either uGRS or wGRS to the model only explained an extra 1.27% of variance of BMI in our RADIANT sample, suggesting that the clinical utility of the GRS is at best modest. Underlining this conclusion further, when the area under (AUC) was calculated on the basis of a receiver operating characteristics (ROC) analysis to discriminate the presence /absence of obesity, the AUC was only 0.58 if only included GRS and ancestry were included in the regression model. The AUC increased to 0.65 when age and sex were included in the model, and it further increased to 0.71 if depression status was also included.

In summary, the findings of Chapter 5 indicated that whilst uGRS or wGRS were normally distributed in both cases and controls, and were significantly associated with higher BMI, both types of GRS explained only a modest proportion of the variance in BMI. As such, whilst molecular markers can at present be used to construct a score that is a significant predictor of obesity, many more markers will need to be identified before we can construct a risk score that has utility clinically. Even adding other non-molecular risk factors, such as age, sex and depression status into the predictive model could not turn it into a useful screening tool.

Question 5: Can Mendelian randomization analysis using instrumental variables disentangle the causal relationship between increased BMI and MDD?

Although it is well established that obesity is overrepresented in individuals with MDD, the causal relationship between increased BMI and MDD remains unclear. Recently, Mendelian randomization (MR), a method that applies genetic information to disentangle the causal relationship between a risk exposure and a disease has been developed. The basic idea of MR analysis is that genetic variants are allocated during meiosis, and are therefore independent of common confounding factors that could contribute to the relationship between the exposure (e.g. high BMI) and the disease (e.g. MDD). Since genetic variants influence the disease outcome only via exposure, MR analysis can provide estimates of association that are free of confounding environmental factors (e.g. stress exposure).

Two instrumental variables (IVs), *FTO* genotype and weighted GRS were used for MR analysis. Both IVs were significantly associated with BMI, suggesting that they are valid indicators of adiposity. BMI was also associated with MDD in the regression analysis. However, our the instrumental variable analyses did not support a causal relationship between higher BMI and major depression, with *FTO* genotype and GRS not acting as significant predictors of MDD. As such, the findings did not support the hypothesis that increased BMI causes clinically significant MDD, whilst applying either *FTO* genotype or wGRS as instrumental variables. However, one limitation of this study is the fact that the study was cross sectional, meaning that

BMI obtained at interview was used as the proxy of premorbid BMI, on the basis that that previous studies have shown that BMI in adolescence is highly associated with BMI in adulthood. Future studies using longitudinal designs are needed to address this limitation. In addition, the negative result could represent either a true negative one or a false negative. One difficulty of conducting Mendelian randomization studies is that no satisfactory formal power calculation methods have yet been developed, meaning that this study may have been underpowered. That said, our sample size is comparable to or larger than those of previous MR studies including one which purported to show a causal relationship between high BMI and depressive symptoms.

Question 6: Can genetic association studies of physical diseases shed light on the co-occurrence between physical illnesses and MDD?

Because as yet, no replicable genetic variants reaching genome-wide significance have been identified in GWAS of MDD (Wray et al., 2012), there is no simple, direct means to determine whether MDD has a molecular genetic overlap with other diseases by looking up overlapping associations. Chapter 7 described several methods to investigate this problem. Two of the approaches identified (the polygenic score approach or GCTA analysis) were not possible due to the lack of availability of genome-wide data on the physical diseases on which my analysis was focused. Therefore, I used publicly available GWAS data on physical diseases to identify the genetic variants reaching genome-wide significance for the physical diseases of interest. I then tested case/ control differences in a large GWAS dataset on MDD. One SNP that is a risk loci consistently associated with asthma (rs1342326, which is

near the *IL33* gene) was significantly over-represented in individuals with MDD. Subsequently, a weighted genetic risk score (wGRS) was constructed for asthma using twelve SNPs identified in published GWAS. However, a further Mendelian randomization (MR) analysis conducted using instrumental variables of risk alleles for rs1342326 and GRS for asthma did not support the hypothesis of a causal relationship between suffering from asthma and developing MDD. Rather, the results suggest a single marker indicating a point of genetic overlap between the liability to develop both MDD and asthma, which might in part contribute to the excess of asthma in individuals with MDD compared to healthy controls. Examinations of the odds ratios derived from our results suggests that variation in rs1342326 explains only part of the asthma/MDD association. The OR for asthma in MDD cases versus controls is 1.79, whereas the OR for the rs1342326/MDD association is 1.15 and the OR for the rs1342326/asthma association is 1.31. Thus, there may be other points of genetic overlap between asthma and MDD and/or other as yet unidentified environmental causes of the asthma/MDD association.

9.2 Clinical implications

The studies presented in this thesis highlight the importance of understanding the co-morbidity between physical diseases and MDD, not only in individuals with MDD, but also in their unaffected relatives. Perhaps the most striking and arguably the most clinically important result is the association between MDD and ‘metabolic syndrome’ (Chapter 3). The term metabolic syndrome has often been criticized as not encompassing a ‘real’ syndrome, but simply a cluster of disorders all related to being

overweight. Certainly, there was good evidence of this clustering in the RADIANT sample, as well as obesity being over-represented in depressed people. In looking for possible reasons for the over representation of physical disorders in MDD generally, the personality trait of neuroticism did not seem to play a major role (Chapter 4). Nor was there evidence of a ‘direction of cause’ effect from high BMI to MDD. Rather there was evidence, at least in the RADIANT sample, of a significant, perhaps substantial genetic correlation between MDD and BMI. This would be in keeping with the finding that some of the disorders in the ‘metabolic syndrome’ category were over-represented not just in MDD sufferers, but also in their unaffected siblings. The other disorder with evidence for a modest genetic overlap with MDD was asthma, which again showed a tendency to be increased in unaffected siblings.

There are two clinical implications from these studies. First, results, when published in academic journals, may serve as a reminder to clinicians (either general practitioners or psychiatrists) to investigate metabolic related physical diseases or their risk factors when diagnosing an individual with MDD. When people report their family history of MDD, clinicians should consider the associated increased risk of various physical diseases. The findings presented here are in line with previous studies (Cuijpers and Smit, 2002, Wulsin et al., 1999), suggesting that there is an increased risk of mortality in depression. Although I did not find gender differences in *morbidity*, previous studies (Wulsin et al., 1999, Joukamaa et al., 2001) have suggested differences in *mortality*; with an increased risk of dying from cardiovascular diseases in depressed men, and an increased risk of dying from respiratory diseases in depressed women.

A second finding with potential clinical implications is the observation of a substantial “SNP heritability” in both MDD and BMI, and a significant genetic correlation between these two diseases/ traits in our RADIANT data. These findings raise the question of whether this observed genetic overlap could help to identify physiological or psychological pathways that might point to treatments that could be applied in a clinical setting. However, the straightforward answer so far is rather disappointingly ‘no’. The analysis did not identify any actual genes that contribute to variance in both MDD and ‘metabolic syndrome’ disorders, and using a GRS approach derived from GWAS of BMI suggested that this score could only explained a modest proportion of variance of BMI, and is not likely to be of any predictive clinical utility. In fact, it could be argued that so far genetic tests generally have been disappointing in providing clinically useful information to inform the prediction of onset of complex diseases, except for those where there are subtypes with a monogenic basis, e.g. Maturity Onset Diabetes of the Young (MODY) (Mitchell, 2012).

With GWAS identifying numerous risk variants responsible for various complex diseases/ traits, the public expects some translation of the genetic findings and the development of novel clinical applications. For example, one important application would be the use of genetic information to identify individuals at risk of developing certain diseases. Unlike monogenic diseases, mounting evidence has shown that multiple genetic and environmental factors contribute to the etiology of complex diseases/ traits. Therefore, genetic information usually provides a continuum of risk

for complex diseases, rather than distinct risk category of risk. Given that environmental factors contribute a substantial proportion of the variance of complex diseases, diagnosis by genetic tests alone is likely to be impractical and unnecessary given that there is a long way to go before all causal genetic variants for any complex disease are identified (Belsky et al., 2013). Since the predictive value is limited for each single genetic variant due to their small effects, using a composite risk assessment such as genetic risk score (GRS) to take account multiple genetic variants simultaneously, also named genomic profiling (Ehret et al., 2011), is an alternative for risk assessment of certain complex diseases. Our findings suggest that using a composite genetic risk assessment is not yet suitable for individual level use, due to low predictive utility. This raises a particular concern, given that recently some commercial companies have begun to advertise genetic testing, including estimation of risk of certain diseases, despite the limited evidence base at present. There seems little doubt that immature genetic prediction based on current knowledge has the potential to cause confusion, unnecessary medical examination or heightened psychological distress (Caulfield et al., 2010). A recent large scale study suggested that genome-wide testing did not cause any short term changes in psychological health, diet or exercise behavior or use of screening tests, although the results are weakened by the lack of a control sample (Bloss et al., 2011). As such, clinicians should inform patients about the limitations of current genetic testing.

9.3 Limitations

Several limitations of the studies described in this thesis need to be addressed. First, these studies were mainly based on secondary analysis of existing datasets were not purpose built for the types of analysis that I aimed to carry out. Thus, for example, I did not have information about smoking, diet and exercise, all of which would have been desirable for comprehensively assessing physical health and its relationship with MDD. Second, most of BMI and physical disease data were based on self-reported information. However, I was able to compare self-report with objectively measured height and weight in a large subset of over 800 participants, which indicated a very high correlation. Similarly a previous study on the DeCC component of the RADIANT data found very good agreement between self-report and general practitioner reports of the presence of physical disorders. A third limitation of these studies is that the DeNT study mainly comprised depressed probands, affected siblings and a small number of unaffected siblings. It would have been desirable to have data on siblings of healthy controls, and more data on unaffected siblings. Lack of such data prevented us from determining with greater confidence the co-familiality of certain physical diseases and depression. Fourth, the study designs were cross-sectional not longitudinal. Therefore, it was difficult to ascertain the causal relationship between the risk factors and the outcomes even after applying Mendelian randomization (MR) analysis.

9.4 Future research directions

Although the association between MDD and various physical diseases, especially metabolic related diseases, has been confirmed in various studies including those

presented here, at present, little is known about the causal underpinnings of the co-morbidity between MDD and physical diseases. I have demonstrated that genetic factors play an important role for both MDD and BMI, and that there may be overlap between such factors. However, it would have been desirable to be able to explore environmental factors that may contribute to the covariance between MDD and BMI to a greater extent. Epidemiological studies have found that childhood adversity and poverty contribute to obesity (Thomas et al., 2008, Drewnowski and Specter, 2004) and MDD (Belle Doucet, 2003, Batten et al., 2004). Gene-environment interactions require exploration in future studies (Belsky et al., 2013). Critical periods for gene environment interactions may also require examination, which would necessitate longitudinal investigation over an extended age range and time period. In addition, the heterogeneity of major depressive disorder, as discussed in Chapter 1, should be considered in future studies, because different phenotypic manifestations may represent different underlying disease-causing mechanisms, which might relate to different physical diseases. It would also be desirable in future studies to include samples derived from and representative of the general population, rather than just clinically ascertained samples such as those considered in this thesis.

The genetic correlation between MDD and BMI suggests that both diseases/ traits share some genetic variants, although we cannot determine which ones are responsible for the overlap between these phenotypes. Increasing the sample size of GWAS data available on both MDD and BMI would facilitate the identification of risk variants. Both MDD and obesity are thought to involve chronic inflammatory processes (Dantzer et al., 2008, Monteiro and Azevedo, 2010). It might therefore be of interest to focus in particular on genetic variants related to pro-inflammatory

makers such TNF-alpha, IL-1, and IL-6. For example a genetic risk score approach for inflammation could be useful in the prediction of co-susceptibility to inflammation-related mental and physical diseases. As discussed in Chapter 8, inflammation is associated with depression and physical disease. However, the true causal factor might be the toxic metabolites, rather than the inflammatory markers per se. Future studies should take account inflammation and associated toxic metabolites to investigate the relationship between major depression and physical diseases. It would also be desirable, as I have already mentioned, to explore genome-wide genetic information for various physical diseases and construct a polygenic risk score to see to what extent this score can explain the variance of MDD. Similarly, the time is ripe to use the bivariate GCTA analysis approaches to estimate genetic correlation between MDD and various physical diseases. For the time being, these ideas will be best pursued through collaborations among research groups with access to large existing data sets. There are however even larger more ambitious studies in progress involving very large cohorts. These include the UK Biobank project, which involves half a million adults of middle / late middle age on whom basic phenotypic data on depression, as well as data on the physical disorders is being acquired. There will soon be GWAS data on the UK Biobank sample, and in the not too distant future whole genome sequence data will become affordable and available on this and other huge datasets.

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